Effects of the Sustained Release of IGF-1 on Extraocular Muscle of the Infant Non-Human Primate: Adaptations at the Effector Organ Level

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PURPOSE. The authors have demonstrated that prolonged exposure of adult rabbit extraocular muscle (EOM) to insulin-like growth factor-1 (IGF-1) results in significantly increased cross-sectional area and muscle force generation lasting over 3 months. Here the authors assess the effects on EOM of sustained IGF-1 treatment on normal binocular infant *Macaca mulatta*.

METHODS. Sustained-release IGF-1 pellets were implanted bilaterally in each medial rectus (MR) muscle of two normal infant non-human primates. Eye position was examined using corneal light reflex testing. After 3 months, morphometric analyses of myofiber cross-sectional area and innervation density in treated MR muscles were compared with an age-matched control and with antagonist lateral rectus (LR) muscles.

RESULTS. After 3 months, the slow-release pellets remained at the implantation site in all four MR muscles treated. The treated MR showed pronounced increases in cross-sectional area and nerve density, mirrored in the untreated antagonist LR.

CONCLUSIONS. Three months of bilateral sustained IGF-1 release in infant non-human primate MR resulted in increased muscle size and innervation density, mirrored in the untreated antagonist LR. It appears that bilateral MR treatment resulted in slow adaptation of both treated MR and contralateral LR muscles over time such that functional homeostasis and near-normal alignment were maintained. Further work is needed to determine what signaling mechanisms maintain proportional innervation when EOMs are forced to adapt to an externally applied perturbation. (*Invest Ophthalmol Vis Sci.* 2012;53:68–75) DOI:10.1167/iovs.11-8356

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Strabismus is a common ocular motor disorder characterized by a misalignment of the eyes, with 3–5% of the children in the United States affected.^{1,2} Patching and glasses are often the first line of treatment, but surgery is required for persistent misalignment to improve the potential for recovering or preserving binocular function. Surgical treatment of strabismus entails either resection of the “underacting” extraocular muscle (EOM), recession of the “overacting” muscle, or a combination of both procedures. Surgical success rates vary, and these depend on the type of strabismus. Surgical success rates also vary if they are characterized by only their motor outcome, that is, eye position, or if both sensory and motor outcomes of surgery are measured. In children, surgical failure rates are reported to be as high as 50%.^{3,4} Botulinum toxin injections are also an effective treatment, although more often when the angle of misalignment is small.⁵ Botulinum toxin only weakens an “overacting” muscle. To parallel the effects of incisional surgery, a pharmacologic treatment is needed that can strengthen an “underacting” muscle. We have shown that injection or sustained release of insulin-like growth factor-1 (IGF-1) in the EOM of adult rabbits effectively increases both muscle cross-sectional area and muscle force generation.^{6–8} Work in developing and juvenile chickens also shows that single orbital injections of IGF-1 increase the strength and mass of treated EOM.^{9,10}

In this study, we sought to determine whether 3 months of sustained release of IGF-1 in the EOM of a non-human primate would result in increased muscle size and alteration of eye position. The macaque monkey has binocular coordination of eye movements similar to that of humans and, to move toward a phase I clinical trial, effectiveness in a similarly binocular visual system is imperative. It is well known that creating strabismus in infant monkeys by surgical manipulation of the EOMs is difficult.¹¹ Most primate models of strabismus involve perturbations of the afferent pathway in the infant monkey, including monocular deprivation¹² or rearing with prism goggles.¹³ Therefore, an additional goal of this study was to determine whether treatment of both medial rectus (MR) muscles would be sufficient to induce strabismus in normal infant monkeys. The results were surprising, based on our previous studies using unilateral IGF-1 and BMP-4 (bone morphogenetic protein 4) treatment in adult rabbits.^{6–8} Sustained IGF-1 treatment over 3 months altered muscle properties in the infant monkey, but essentially did not alter functional eye alignment. These results suggest that ongoing processes of muscle and innervational adaptation play an important role in the maintenance of binocular fusion.

METHODS

Macaca mulatta monkeys aged 1 to 2 weeks were obtained from the breeding colony at the Yerkes National Primate Research Center,

Emory University in Atlanta, Georgia. All experiments were approved by the Emory University Animal Care Committee, and followed the National Institutes of Health and ARVO guidelines for use of animals in research. The infant monkeys were photographed to document orthotropic eye alignment before treatment, and all were normal. The pellets, measuring 3×1.5 mm, released $1 \mu\text{g}$ IGF-1/d for a total of 90 days (Innovative Research of America, Sarasota, FL). This release rate was based on a large number of previous studies examining dose effects from single injections in rabbits^{6,7} and the effect of pellets on rabbit EOM.⁸ Before implantation, pellets containing IGF-1 were gas sterilized. Under general anesthesia, an incision was made in the medial conjunctiva, and the MR muscles were visualized surgically. The muscles were retracted with a small muscle hook at their point of insertion, and the pellets were placed in Tenon's capsule overlying each MR muscle. The conjunctiva was closed with 8-0 ophthalmic suture. The infants were monitored visually for postimplant alignment and eye motility. Eye alignment was assessed by corneal light reflexes, which are relatively imprecise. Essentially no change in alignment was detected and confirmed by two independent observers. In addition, we obtained the rectus muscles from a 3-month-old macaque that had to be euthanized due to a trauma unrelated to the head. These muscles served as age-matched controls.

Three months after implantation, the monkeys were euthanized by staff veterinarians at the Yerkes Primate Center. All extraocular muscles were dissected from origin to insertion, embedded in tragacanth gum, and frozen on 2-methylbutane chilled to a slurry on liquid nitrogen. The tissue was stored at -80°C until processed. The muscles were sectioned at $12 \mu\text{m}$ in a cryostat and processed for immunohistochemical visualization of fast, slow, developmental, and neonatal myosin heavy chain (MyHC) isoforms (fast and slow, 1:40; developmental and neonatal, 1:20; Vector Laboratories, Burlingame, CA). In addition, sections were selected to represent regions near the entry zone of the oculomotor nerve division into the muscles, as well as sections toward the tendon end of the muscle, and immunostained for the presence of myelinated nerve fibers using an antibody to Schwann cell myelin (1:50; Cosmo Bio Co., Tokyo, Japan) or neurofilament (1:1000; Covance, Princeton, NJ). The sections were incubated in reagents from a commercial kit (Vectastain Elite ABC kit; Vector Laboratories) and reacted using the diaminobenzidine procedure intensified by the addition of cobalt chloride and nickel ammonium sulfate solutions. Sections immunostained for the MyHC isoforms were analyzed for mean myofiber cross-sectional area and percentage of myofibers positive for each of the individual MyHC isoforms based on total fiber number. Three slides each were analyzed for each immunostain in the midregion of the rectus muscles and in the tendon region. Slides were chosen by determining distance from the tendon end of all muscle fibers, as well as close examination of fast MyHC isoform expression patterns, which are illustrative of position along the muscle length. A total of three to five fields were counted to count a minimum of 200 myofibers in both the orbital and global layers per stained slide, using the central region of each muscle section to avoid potential edge effects. In addition, total myofiber number was determined by counting every myofiber in whole muscle cross-sections, keeping the counts of orbital and global layers separate. Minimally, three slides were counted in their entirety, for the control MR and for all four IGF-1-treated MR. Schwann cell myelin and neurofilament-stained material were analyzed to measure the area occupied by nerve bundles (in μm^2) positive for myelinated axons as a ratio of total muscle area (in μm^2) per microscope field examined both for the tendon and midbelly regions. A total of three to five fields were examined per region per muscle section. Data are presented as mean $\pm$ SEM. All quantification was performed using imaging analysis software (BioQuant Nova Prime morphometry program; BioQuant, Nashville, TN).

RESULTS

After 3 months of sustained IGF-1 treatment to the MR muscles, the pellets were in position over each of the treated MR

muscles (Fig. 1). Mean muscle cross-sectional areas of global layer fibers were larger in the MR muscles after 3 months of IGF-1 treatment compared with the age-matched control muscles (Fig. 2). In the treated MR, these fiber changes were most pronounced in the global layer in both the midregion and tendon ends of both the treated MR muscles. The antagonist lateral rectus (LR) muscles also had noticeably larger mean cross-sectional areas in the global layer compared with controls (Fig. 2). In the global middle and tendon regions, cross-sectional areas increased approximately 2-fold in the treated MR muscles and 2.5-fold in the untreated antagonist LR muscles. It is unlikely that changes in the LR muscle were due to unintended diffusion of IGF-1 within the orbit, in part due to the extremely low release rate of IGF-1 from the pellets and potentially the confinement of the pellet within the connective tissue sheaths of the MR muscles. To test this, the superior (SR) and inferior (IR) rectus muscles were analyzed to determine whether changes in the contralateral LR could be due to diffusion of drug (Fig. 2). The mean cross-sectional myofiber areas of both the SR and IR muscles were similar to the control MR muscles. Thus, it is unlikely that changes in the LR were due to "diffusion" of IGF-1 within the orbit. To determine whether the IGF-1 resulted in hyperplasia, all myofibers were counted in the control and IGF-1-treated EOM, and no differences were seen in total counts (Fig. 3) or when analyzed as fiber number in the orbital or global layers (Fig. 3).

The effect of the sustained IGF-1 treatment on innervation was also assessed. Myelinated nerve fiber densities in the treated MR muscles were increased 2-fold in both the midregion and tendon ends of the muscles (Fig. 4). The contralateral antagonist LR muscles showed a 50-fold increase in myelinated nerve density in the tendon region of the muscles. In addition, examination of the density of neurofilament-positive axons showed a relatively similar picture (Fig. 5). This analysis also points out some pronounced differences in nerve density between control MR and LR muscles. Although the overall nerve density is similar in the middle regions of both muscles, there is a 25% greater nerve density in the tendon region of the MR muscles. Because this is normalized to muscle area, this difference must be based on functional differences in this region of the MR muscle compared with the LR.

IGF-1 plays a role in muscle growth, so changes in the population of myofibers expressing the two "immature" MyHC isoforms were examined. In the midregion of the treated MR muscles, there was a large decrease in the percentage of myofibers expressing embryonic MyHC (Fig. 6). A similar trend

FIGURE 1. Magnetic resonance imaging view of IGF-1 pellets in place on the MR muscles bilaterally. Images were acquired with a 3-Tesla magnet (GE) and viewed with NeuroLens software.

FIGURE 2. Three months of sustained-release of IGF-1 results in increased myofiber cross-sectional area of the treated MR muscles. (A) Control MR myofibers in the global layer immunostained for the presence of fast MyHC isoform. (B) IGF-1-treated MR myofibers in the global layer immunostained for the presence of fast MyHC isoform. Bar: 100 μm. (C–F) Quantification of mean myofiber cross-sectional areas after 3 months of sustained IGF-1 treatment in the orbital (C, D) and global (E, F) layers in the middle of the muscle (C, E) and tendon regions (D, F). IR, inferior rectus; SR, superior rectus.

was seen in the tendon region of the treated MR. In contrast, in the untreated antagonist LR muscles there was an increase in the proportion of embryonic MyHC positive myofibers (Fig. 6). In the treated MR muscles, the global layer increased its expression of neonatal MyHC, whereas the orbital layer was either unchanged (midregion) or decreased (tendon region) (Fig. 7). The levels of neonatal MyHC expression in the LR muscles were very limited in these specimens. No changes were seen in the percentage of myofibers expressing fast MyHC in any of the muscles examined (data not shown).

DISCUSSION

Bilateral treatment of the MR muscles in infant primates with sustained release of IGF-1 for 3 months resulted in changes in myofiber cross-sectional area and innervational density in the treated muscles. The nontreated antagonist LR muscles were comparably altered bilaterally. Area measurements in the IR and SR of the treated monkeys were similar to those measured

in the control MR muscles; thus, it is unlikely that changes in the LR were due to “diffusion” of IGF-1 within the orbit. Compensatory changes have long been hypothesized to play a role in determining the response to strabismus treatment.¹⁴ Previous studies have demonstrated a significant increase in muscle force generation, both as measured in grams and as specific tension (force/muscle cross-sectional area).^{6–8} It is possible, however, that a significantly larger daily dose of IGF-1 might be able to override binocular fusion sufficiently to alter eye position because it is unknown what dose is needed to saturate all IGF-1 receptors in the infant EOM, and receptor turnover and upregulation with increased exposure to exogenously added IGF-1 may occur. It may also be that doses will vary significantly in adults compared with the infants studied here. Future experiments will address this.

It is striking that the size ratio difference between the LR and MR muscles is relatively unaltered compared with control in these muscles after 3 months of continuous MR treatment with IGF-1. Myelinated nerve density increased after the sus-

FIGURE 3. (A) Total myofiber number in control and IGF-1-treated MR muscles. (B) Total myofiber number in the orbital and global layers of control and IGF-1-treated muscles.

tained IGF-1 treatment. Interestingly, there was a 2-fold increase in nerve density in the treated MR, whereas in the antagonist LR there was a 50-fold increase in nerve density. This is quite remarkable because the LR muscles were not exposed to exogenous IGF-1. These results suggest that pronounced adaptations occur in the antagonist LR as a consequence of sustained exogenous IGF-1 dosing to both MR muscles. These adaptations may result from increased resting tension in the LR muscles as a consequence of bigger, stronger MR muscles, or they could result from central adaptations in the version and vergence systems that maintain alignment.

It is interesting that only minimal changes were seen in MyHC isoform expression patterns except for embryonic MyHC. These changes were inversely related in the MR and LR muscles, with decreased percentages of embryonic MyHC-containing fibers in the treated MR and increases in the contralateral but untreated LR muscles. No changes were seen in fast MyHC isoform expression. In an earlier study, we showed that 1 week after a single injection of IGF-1 in rabbit SR muscle, the percentage of positive myofibers for both embryonic and neonatal MyHC isoforms decreased, with concomitant reductions in shortening velocity.⁷ Future studies will include examination of muscle force and contractile characteristics in the treated non-human primate muscles.

IGF exerts an important role in muscle development and growth.^{15,16} It is downregulated in adult noncranial skeletal muscle, but is reexpressed after muscle injury, playing a role in

facilitating muscle regeneration.¹⁷ There is a great deal of literature supporting the ability of exogenously added IGF-1 to increase muscle size and force generation in diseased and aging skeletal muscle.^{18–20} Although we recently demonstrated that there is no correlation between myofiber diameter and force generation in single isolated normal adult EOM myofibers,²¹ previous studies using both acute^{6,9} and long-term IGF treatment⁷ demonstrated that muscle force generation is significantly increased by this treatment. In the present study, the increase in mean myofiber cross-sectional area in the treated MR muscles supports this view. However, the coordinated changes in the antagonist muscles we observed could have the effect of maintaining normal alignment in these treated infant monkeys. It was possible that myofiber hypertrophy induced by IGF-1 treatment could also be accompanied by myofiber hyperplasia. This question was answered by examination of the mean of the total number of myofibers in control and IGF-1-treated MR that did not appear to differ, suggesting new fiber formation did not occur. This is supported by the lack of increased numbers of myofibers expressing the immature MyHC isoforms, and by our previous study showing that myofiber remodeling is not correlated with increased numbers of neonatal MyHC-expressing myofibers.²²

The growth-promoting effect of IGF-1 on nerve outgrowth is also well established. For example, exogenous addition of IGF-1 or -2 results in intramuscular nerve sprouting in normal adult skeletal muscle.²³ When applied to motor nerves, rapid axoplasmic transport occurs, where it presumably exerts trophic influence on the motor neurons.²⁴ During motor nerve regeneration supplying adult muscles, where expression is downregulated, increased expression of IGF-1 was temporally correlated with nerve growth.²⁵ IGF-1 promotes motor neuron survival in developing oculomotor neurons, and exogenous treatment increases neurite outgrowth in vitro.²⁶ Thus, the increase in nerve density after 3 months of sustained IGF-1 treatment would be an expected sequela. However, the similar and coordinated increase in the nerve density in the untreated LR muscles was not expected. The LR muscle is innervated by the motor neurons in the abducens nucleus, whereas the MR is innervated by the oculomotor nucleus. These results suggest a robust ability of the ocular motor system to communicate alterations of properties in the treated neurons to the untreated motor neurons of the antagonist muscle. The results of our study do not point to a clear mechanism, and experiments are under way to elucidate this.

Detection of binocular visual error by the cortical system may have led to ocular motor adaptations, including changes in muscle innervation and contractility. Centrally, communication of changes between yoked muscle-neuronal pairs may have occurred. A potential candidate for this internuclear communication is the abducens interneuron population, projecting directly from the abducens motor nucleus to the contralateral oculomotor nucleus.²⁷ Retrogradely transported growth factors have been shown to alter contractile properties in treated motor units. For example, after axotomy of the abducens nerve, exogenously added neurotrophin-3 or brain-derived neurotrophic factor each restore different aspects of firing rate characteristics in abducens motor neurons.²⁸ We have previously shown that treatment of rabbit EOM with IGF-1 alters the peak rate of force development.⁷ This alteration in contractility is most likely due to alteration in motor neuron firing rate.²⁹ Recent work has confirmed these findings in juvenile chick EOM.³⁰ Future work will attempt to address the potential mechanism(s) of the communication to the motor nucleus of the antagonist, yet untreated, muscle.

This bilateral sustained IGF-1 treatment, although causing both increased innervation to the muscles and increased myofiber size, did not result in the development of a strabismus.

FIGURE 4. Three months of sustained release of IGF-1 results in increased myelinated nerve density per muscle area in the treated MR muscles. Each photomicrograph shows global layer on the *left side* and orbital layer on the *right side*. (A) Control MR myofibers immunostained for the presence of myelinated nerve using an antibody specific for Schwann cells. (B) IGF-1-treated MR muscle. Bar: 100 μm . (C, D) Quantification of myelinated nerve density/myofiber cross-sectional area after 3 months of sustained IGF-1 treatment in (C) the midregion of the muscle and (D) in the tendon region.

FIGURE 5. Quantification of total axon density/myofiber cross-sectional area after 3 months of sustained IGF-1 treatment in the mid-region (A) and the tendon region (B).

This was not an unexpected result because it is well known that the brain maintenance of binocularity is extremely robust in normals.³¹ Primate models of experimentally induced strabismus all involve significant afferent perturbations, including prolonged alternating monocular occlusion,^{32,33} induced anisometropia,³⁴ or prism goggles.¹³ In an otherwise normal monkey, our results support the concept that the normal ocular motor system can adapt to changes of slow onset and retain binocular coordination by generating similar changes in antagonist muscles.

Although the factors that allow maintenance of binocular alignment in this model are likely complex and are not yet worked out, there are two most likely mechanisms. The first invokes Hering's law of equal innervation, a mechanism proposed to account for eye movement conjugacy. Hering's law postulates that to maintain conjugate gaze, the brain sends a similar command to yoked muscles. For vertical eye movements, a structural substrate does exist: premotor neurons project to yoked muscles of both eyes.³⁵ For horizontal eye movements, the picture is more complex. Internuclear neurons between the abducens nucleus and the oculomotor nuclei allow coactivation of synergistic muscles. Second, a strong body of research suggests that a large population of cranial motor neurons to the EOM selectively provide unocular eye commands.³⁶ Although the brain sends specific signals for the left and right eyes, Hering may still be partly right, in that the oculomotor system has innate systems that control and maintain conjugacy of both eyes. Evidence suggests that the poste-

FIGURE 6. Quantification of embryonic MyHC isoform expression after 3 months of sustained IGF-1 treatment. # indicates data not included. Exp, experimental LR opposite to a treated MR.

rior parietal cortex controls binocular coordination of saccades,³⁷ and that adaptations restore functional yoking of eyes to preserve conjugate gaze.³⁸ Our data demonstrate distinct properties relative to the innervation of the muscles themselves in relation to Hering's law. First, although nerve density is similar in the midregion of both the control MR and LR, within the tendon region a striking difference in innervation density is seen. This could be due to different myofiber numbers in the LR and MR muscles,³⁹ different proportions of

multiply innervated myofibers in these two muscles,⁴⁰ the presence of greater numbers of short fibers within the MR,⁴¹ or a combination of all these factors. Thus, "equal innervation" is more aptly called "proportional innervation," and this proportionality must be maintained to retain conjugate gaze. After 3 months of sustained IGF-1 treatment, in the tendon region of the treated MR muscles innervation density increased 2-fold, although there was a 50-fold increase in the tendon region of the LR muscles in the orbit with the treated MR muscles (see

FIGURE 7. Quantification of neonatal MyHC isoform expression after 3 months of sustained IGF-1 treatment.

FIGURE 8. Schematic model depicting potential pathways for communication of changes in the treated MR muscles to the abducens motor nucleus, in turn increasing nerve outgrowth into the contralateral but untreated LR.

Figs. 4 and 5). The explanation for this difference is unclear. However, it supports the view that feedback of the imposed changes in the treated MRs was communicated to the brain, resulting in LR adaptation. One hypothesis is that abducens internuclear neuronal signaling that is provided to the yoked muscles controls this process (Fig. 8). Although further studies are needed to dissect out the potential CNS mechanisms that control these adaptations, one has to assume that the coordinated nerve outgrowth in the untreated LR muscles was due to an active process. It is interesting to point out, however, that the same treatment given to an adult strabismic monkey, in that case being administered unilaterally, resulted in large bilateral reduction in the angle of eye deviation in primary gaze (McLoon LK, et al., unpublished data, 2010).

Another possible mechanism for maintenance of alignment in this model is an increase in divergence tone in response to the convergent error associated with treatment-related changes in the MR muscles of each eye. To maintain binocularity in the setting of bilaterally stronger, bigger MR muscles, active divergence would be required. These innervational changes may come in the form of increased neuronal firing rate, an increase in innervational density, or, more likely, both. Irrespective of whether the mechanism is principally driven by the vergence systems, the end result is maintenance of binocularity.

In summary, 3 months of sustained release of IGF-1 delivered to both MR muscles in infant monkeys resulted in pronounced bilateral increases in the mean cross-sectional areas of myofibers and in nerve density in both the treated MR muscles and the antagonist but untreated LR muscles. Central and peripheral adaptive mechanisms provide sufficient ocular motor plasticity, especially in an infant non-human primate model, to compensate for slow growth induced by sustained low levels of IGF-1 treatment of the medial rectus muscles.

References

- Greenberg AE, Mohny BG, Diehl NN, Burke JP. Incidence and type of childhood esotropia: a population-based study. *Ophthalmology*. 2007;114:170–174.
- Louwagie CR, Diehl NN, Greenberg AE, Mohny BG. Is the incidence of infantile esotropia declining? A population-based study from Olmsted County, Minnesota. 1965–1994. *Arch Ophthalmol*. 2009;127:200–203.
- Vroman DT, Hutchinson AK, Saunders RA, Wilson ME. Two-muscle surgery for congenital esotropia: rate of reoperation in patients with small versus large angles of deviation. *J Am Assoc Pediatr Ophthalmol Strabismus*. 2000;4:267–270.
- Pineles SL, Ela-Dalman N, Zvansky AG, Yu F, Rosenbaum AL. Long-term results of the surgical management of intermittent exotropia. *J Am Assoc Pediatr Ophthalmol Strabismus*. 2010;14:298–304.
- Rayner SA, Hollick EJ, Lee JP. Botulinum toxin in childhood strabismus. *Strabismus*. 1999;7:103–111.
- McLoon LK, Christiansen SP. Increasing extraocular muscle strength with insulin-like growth factor II. *Invest Ophthalmol Vis Sci*. 2003;44:3866–3872.
- Anderson BC, Christiansen SP, Grandt S, Grange RW, McLoon LK. Increased extraocular muscle strength with direct injection of insulin-like growth factor-I. *Invest Ophthalmol Vis Sci*. 2006;47:2461–2467.
- McLoon LK, Anderson BC, Christiansen SP. Increasing muscle strength as a treatment for strabismus: sustained release of insulin growth factor-1 results in stronger extraocular muscle. *J Am Assoc Pediatr Ophthalmol Strabismus*. 2006;10:424–429.
- Chen J, von Bartheld CS. Role of exogenous and endogenous trophic factors in the regulation of extraocular muscle strength during development. *Invest Ophthalmol Vis Sci*. 2004;45:3538–3545.
- Li T, Wiggins LM, von Bartheld CS. Insulin-like growth factor-1 and cardiotrophin 1 increase strength and mass of extraocular muscle in juvenile chicken. *Invest Ophthalmol Vis Sci*. 2010;51:2479–2486.
- Hubel DH, Wiesel TN. Binocular interaction in striate cortex of kittens reared with artificial squint. *J Neurophysiol*. 1965;28:1041–1059.
- Quick MW, Tigges M, Gammon JA, Boothe RG. Early abnormal visual experience induces strabismus in infant monkeys. *Invest Ophthalmol Vis Sci*. 1989;30:1012–1017.
- Chino YM, Smith EL, Wada H, Ridder WH, Langston AL, Leshner GA. Disruption of binocularly correlated signals alters the postnatal development of spatial properties in cat striate cortical neurons. *J Neurophysiol*. 1991;65:841–859.
- Kushner BJ, Vrabec M. Theoretical effects of surgery on length tension relationships in extraocular muscles. *J Am Assoc Pediatr Ophthalmol Strabismus*. 1987;24:126–131.
- Florini JR, Ewton DZ, Falen SL, Van Wyk JJ. Biphasic concentration dependency of stimulation of myoblast differentiation by somatomedins. *Am J Physiol Cell Physiol*. 1986;250:C771–C778.
- Tollefsen SE, Lajara R, McCusker RH, Clemmons DR, Rotwein P. Insulin-like growth factors (IGF) in muscle development. Expression of IGF-1, the IGF-1 receptor, and an IGF binding protein during myoblast differentiation. *J Biol Chem*. 1989;264:13810–13817.
- Jennische E, Hansson HA. Regenerating skeletal muscle cells express insulin-like growth factor I. *Acta Physiol Scand*. 1987;130:327–332.
- Barton-Davis ER, Shotoruma DI, Musaro A, Rosenthal N, Sweeney HL. Viral mediated expression of insulin-like growth factor 1 blocks the aging-related loss of skeletal muscle function. *Proc Natl Acad Sci USA*. 1998;95:15603–15607.
- Lynch GS, Cuffe SA, Plant DR, Gregorevic P. IGF-1 treatment improves the functional properties of fast- and slow-twitch skeletal muscles from dystrophic mice. *Neuromuscul Disord*. 2001;11:260–268.
- Payne AM, Zheng Z, Messi ML, Milligan CE, Gonzalez E, Delbono O. Motor neuron targeting of IGF-1 prevents specific force decline in ageing mouse muscle. *J Physiol*. 2006;570:283–294.
- McLoon LK, Park H, Kim JH, Pedrosa-Domellöf F, Thompson LV. Force, shortening velocity and patterns of myosin heavy chain

- co-localization in single skinned myofibers from adult rabbit extraocular muscle. *J Appl Physiol*. 2011;111:1178-1189.
22. Christiansen SP, McLoon LK. The effect of resection on satellite cell activity in rabbit extraocular muscle. *Invest Ophthalmol Vis Sci*. 2006;47:605-613.
 23. Caroni P, Grandes P. Nerve sprouting in innervated adult skeletal muscle induced by exposure to elevated levels of insulin-like growth factors. *J Cell Biol*. 1990;110:1307-1317.
 24. Hansson HA, Rozell B, Skottner A. Rapid axoplasmic transport of insulin-like growth factor-1 in the sciatic nerve of adult rats. *Cell Tissue Res*. 1987;247:241-247.
 25. Gehrman J, Yao DL, Bonetti B, et al. Expression of insulin-like growth factor-1 and related peptides during motoneuron regeneration. *Exp Neurol*. 1994;128:202-210.
 26. Rind HB, von Bartheld CS. Target-derived cardiotrophin-1 and insulin-like growth factor-1 promote neurite growth and survival of developing oculomotor neurons. *Mol Cell Neurosci*. 2002;19:58-71.
 27. Buettner-Ennever JA. The extraocular motor nuclei: organization and functional neuroanatomy. *Prog Brain Res*. 2006;151:95-125.
 28. Davis-Lopez de Carrizosa MA, Morado-Diaz CJ, Tena JJ, et al. Complementary actions of BDNF and neurotrophin-3 on the firing patterns and synaptic composition of motoneurons. *J Neurosci*. 2009;29:575-587.
 29. Davis-Lopez de Carrizosa MA, Morado-Diaz CJ, Miller JM, de la Cruz RR, Pastor AM. Dual encoding of muscle tension and eye position by abducens motoneurons. *J Neurosci*. 2011;31:2271-2279.
 30. Li T, Feng CY, von Bartheld CS. How to make rapid eye movements "rapid": the role of growth factors for muscle contractile properties. *Pflügers Arch*. 2011;461:373-386.
 31. Takagi M, Trillenberg P, Zee DS. Adaptive control of pursuit, vergence, and eye torsion in humans: basic and clinical implications. *Vision Res*. 2001;41:3331-3344.
 32. Tychsen L, Burkhalter A. Nasotemporal asymmetries in V1: ocular dominance columns of infant, adult, and strabismic macaque monkeys. *J Comp Neurol*. 1997;388:32-46.
 33. Das VE, Mustari MJ. Correlation of cross-axis eye movements and motoneuron activity in non-human primates with "A" pattern strabismus. *Invest Ophthalmol Vis Sci*. 2007;48:665-674.
 34. Kiorpes L, Kiper DC, O'Keefe JP, Cavanaugh JR, Movshon JA. Neuronal correlates of amblyopia in the visual cortex of macaque monkeys with experimental strabismus and anisometropia. *J Neurosci*. 1998;18:6411-6424.
 35. Moschovakis AK, Scudder CA, Highstein SM. A structural basis for Hering's law: projections to extraocular motoneurons. *Science*. 1990;248:1118-1119.
 36. King WM. Binocular coordination of eye movements: Hering's Law of equal innervation or uniocular control? *Eur J Neurosci*. 2011;33:2139-2146.
 37. Vernet M, Yang Q, Daunys G, Orssaud C, Eggert T, Kapoula Z. How the brain obeys Hering's law: a TMS study of the posterior parietal cortex. *Invest Ophthalmol Vis Sci*. 2008;49:230-237.
 38. Maxwell GF, Lemij HG, Collewyn H. Conjugacy of saccades in deep amblyopia. *Invest Ophthalmol Vis Sci*. 1995;36:2514-2522.
 39. Oh SY, Poukens V, Demer JL. Quantitative analysis of rectus extraocular muscle layers in monkey and humans. *Invest Ophthalmol Vis Sci*. 2001;42:10-16.
 40. Mayr R, Gottschall J, Gruber H, Neuhuber W. Internal structure of cat extraocular muscle. *Anat Embryol*. 1975;148:25-34.
 41. Harrison AR, Anderson BC, Thompson LV, McLoon LK. Myofiber length and three-dimensional localization of neuromuscular junctions in normal and botulinum toxin treated adult extraocular muscles. *Invest Ophthalmol Vis Sci*. 2007;48:3594-3601.