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Examination of Feline Extraocular Motoneuron Pools as a Function of Muscle Fiber Innervation Type and Muscle Layer

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Abstract

This study explores two points related to the pattern of innervation of the extraocular muscles. First, species differences exist in the location of the motoneurons supplying multiply innervated fibers (MIFs) and singly innervated fibers (SIFs) in eye muscles. MIF motoneurons are located outside the extraocular nuclei in primates, but are intermixed with SIF motoneurons within rat extraocular nuclei. To test whether this difference is related to visual capacity and frontal placement of eyes, we injected retrograde tracers into the medial rectus muscle of the cat, a highly visual nonprimate with frontally placed eyes. Distal injections labeled smaller MIF motoneurons located ventrolaterally and rostrally within the oculomotor nucleus (III). More central injections also labeled a separate population of larger cells located dorsally in III. Thus, the cat shares with the nocturnal rat the feature of having MIF motoneurons located within the bounds of III. On the other hand, just as with monkeys, cats show segregation of the MIF and SIF medial rectus motoneuron pools, albeit in a different pattern. Second, extraocular muscles are divided into two layers; the inner, global layer inserts into the sclera, and the outer, orbital layer inserts into the connective tissue pulley. To test whether these layers are supplied by anatomically discrete motoneuron pools, we injected tracer into the orbital layer of the cat lateral rectus muscle. No evidence of either morphological or distributional differences was found, suggesting that the

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CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest.

ROLE OF AUTHORS

All authors had full access to all data in the study and take responsibility for the integrity of the data and accuracy of the data analysis. Study concept and design: MOB, SW, MJM, PJM. Acquisition of data: MOB, SW, PJM. Analysis and interpretation of the data: MOB, SW, PJM. Drafting the manuscript: MOB. Critical revision of the article for important intellectual content: MOB, SW, MJM, PJM. Statistical analysis: MOB. Obtained funding: MJM, PJM. Study supervision: SW, PJM.

functional differences in these layers may be due mainly to their orbital anatomy, not their innervation. *J. Comp. Neurol.* 000:000–000, 2016.

INDEXING TERMS

oculomotor; abducens; eye muscle; gaze; eye movements

Coordinated eye movements are used to direct the eyes to targets of interest and to maintain this relationship in the face of external perturbations. This system has been used as a model to study motor control because of our capacity to measure eye movements precisely, the simplicity of its anatomy (only six extraocular muscles per eye), a relatively constant load during eye movements and because the central anatomical connections controlling eye movements are well established. Despite these points, the etiology of eye movement disorders, such as strabismus, is not well understood (Leigh and Zee, 2015). To address this problem better, it is critical that we fully understand the anatomy of both the oculomotor plant and the circuits directing it. One challenge to achieving this understanding is the complexity of the extraocular muscles. The fibers in these muscles are highly specialized, with a wide range of biochemical and morphological fiber subtypes present in each extraocular muscle. Furthermore, they are differentially arranged in two layers (Spencer and Porter, 2006).

Mammalian extraocular muscle innervation patterns are idiosyncratic compared with most skeletal muscles because there are two different patterns by which their fibers receive axonal input from motoneurons. The most common extraocular muscle fiber class is the singly innervated fiber (SIF). Each SIF is contacted by a large-diameter, heavily myelinated axon that forms a single en plaque endplate, which is typical in most skeletal muscles. These endplates form a band across the middle one-third of the muscle (Büttner-Ennever et al., 2001; Namba et al., 1968a,b). Excitation of the SIF axons by electrical or pharmacological means produces an all-or-nothing response in the muscle fiber, resulting in a twitch (Bach-y-Rita and Ito, 1966; Bach-y-Rita et al., 1977; Bondi and Chiarandini, 1983; Chiarandini, 1976; Chiarandini and Davidowitz, 1979; Jacoby et al., 1989). Based on these properties and their histochemical and ultrastructural features, SIFs are categorized as fast-twitch, relatively fatigueable, large-diameter fibers (Spencer and Porter, 2006). The second, less common class is the multiply innervated fiber (MIF). In this case, a thin axon supplies the target fiber with numerous, small bouton terminals organized in an en passant and, occasionally, an en grappe manner. These boutons are distributed along the length of each muscle fiber from origin to insertion (Büttner-Ennever and Horn, 2002; Büttner-Ennever et al., 2001; Eberhorn et al., 2006; Namba et al., 1968a,b). Excitation of MIF axons is believed to produce a slow, graded response in the muscle fiber (Nelson et al., 1986). Given their physiological, histochemical, and ultrastructural features, MIFs are categorized as nontwitch, highly fatigue-resistant, small-diameter fibers (Bach-y-Rita and Ito, 1966; Bach-y-Rita et al., 1977; Bondi and Chiarandini, 1983; Chiarandini, 1976; Chiarandini and Davidowitz, 1979; Jacoby et al., 1989; Spencer and Porter, 2006). This type of fiber is rare in mammals, but has been reported for other small muscles, including the stapedius, tensor tympani, vocalis, and

pharyngeal muscles (for reviews see Morgan and Proske, 1984; Schiaffino and Regioni, 2011).

Büttner-Ennever and Akert (1981) and Porter et al. (1983) used retrograde tracers to label separate, muscle-specific pools of motoneurons in the monkey oculomotor nucleus (III) and noted that, in some cases, more than one pool was labeled for a specific muscle. More recently, Büttner-Ennever and colleagues (2001) used tracer injections into the distal portion of the muscle to limit tracer uptake to the axons supplying MIFs. They found that MIF motoneuronal populations in monkeys were anatomically segregated from the SIF motoneuronal populations. Specifically, MIF motoneurons are smaller cells peripheral to the cytoarchitectonically defined extraocular motor nuclei, whereas SIF motoneurons are larger cells residing within them (Büttner-Ennever and Horn, 2002; Büttner-Ennever et al., 2001; Wasicky et al., 2004). Anatomical distinctions between MIF and SIF motoneurons were further reinforced by immunohistochemical evidence. Specifically, primate SIF motoneurons 1) contain nonphosphorylated neurofilaments, 2) are parvalbumin positive, and 3) are surrounded by perineuronal nets. In contrast, MIF motoneurons displayed no immunoreactivity to these markers (Eberhorn et al., 2005a).

Using the same approach, Eberhorn and colleagues (2006) examined rat oculomotor nuclei to determine whether MIF and SIF motoneurons displayed characteristics similar to those of primates. Just as with monkeys, tracer experiments combined with immunohistochemistry reliably distinguished MIF and SIF populations of motoneurons, and, just as with primates, rat MIF motoneurons were smaller compared with SIF motoneurons. However, in marked contrast to primate oculomotor nuclei, rats generally lacked clear topographic segregation of the two motoneuronal populations; both SIF and MIF cells were found within the nuclei (Eberhorn et al., 2006).

The anatomical segregation of the two extraocular muscle pools in primates may result from the greater demands for oculomotor control in these species. Primates have frontally placed eyes and foveas, and they routinely align both eyes (motor fusion) to examine points of interest. Furthermore, the primate visual system is the dominant sensory input and is critical for foraging, hunting, and surveying the environment for potential threats, all behaviors essential for survival. In contrast, rats are laterally eyed and afoveate. For rats, visual sensory information is secondary to somatosensory and olfactory cues (Anjum et al., 2006; Hughes, 1977; Wallace et al., 2002), so there is less specificity in visual acuity and less demand for precision when it comes to ocular motility in these rodents (Wallace et al., 2013). To test this view of the evolution of MIF motoneuron organization, the current study investigates the organization of III in cats. Although cats lack a fovea, they do have a highly sensitive area centralis. Frontally placed eyes and accurate vision play central roles in many feline behaviors (Hughes, 1975). Because cats possess oculomotility repertoires similar to those of primates, an anatomical organization of MIF and SIF motoneurons closely paralleling those of primates, as opposed to rats, would be expected for cats.

A second unique feature of extraocular muscles is the bilaminar organization of the muscles. Each muscle has an inner, global layer and an outer, orbital layer. These layers differ in their muscle fiber constituents (Oh et al., 2001a,b; Spencer and Porter, 2006) and display other

histological specializations. As each rectus muscle projects anteriorly from its origin, it becomes ensheathed in a collagen and elastin sleeve. Anterior to the equator of the globe in Tenon's fascia, this connective tissue sleeve tethers each extraocular muscle to the bony orbit (Demer et al., 1995), holding the muscle off the globe and functioning as a pulley. The outer, orbital muscle layer inserts into this collagenous connective tissue ring (Kono et al., 2005; Lim et al., 2007; Ruskell et al., 2005; Shin et al., 2012). In contrast, the inner, global layer continues on through the pulley to insert onto the sclera of the eye.

A proposal for describing the interaction between the connective tissue sheathes and their resultant effect on the muscle paths has been put forth, termed the *fibro-muscular pulley hypothesis* (Demer et al., 1995). This hypothesis suggests that pulleys have noteworthy implications for ocular motility. For example, the presence of fibromuscular pulleys contradicts the notion that the vector of action of the rectus muscles begins at the annulus of Zinn. Instead, it originates from the point at which each muscle exits from its pulley. Furthermore, it is likely that the pulleys prevent slippage when the muscle is in secondary and tertiary gaze positions (Clark et al., 1997). This hypothesis has been supported by functional imaging studies (Miller, 1989; Miller and Robins, 1987; Miller et al., 1993). Additionally, the pulleys may prevent these muscles from compressing the globe during contraction, thereby avoiding distortion of the visual receptive surface when the eyes are not centered (Clark et al., 1997; Miller, 1989).

During magnetic resonance imaging scans of behaving humans, Demer and colleagues (2000) noted that the pulleys are not fixed but are instead pulled posteriorly during contraction of the associated muscle (Demer, 2002; Kono et al., 2002a,b; Oh et al., 2001b). This observation conforms to the active pulley hypothesis put forth by Demer (2002) positing that the orbital layer can control the location of the pulley. Thus, the orbital layer may modify the pulling direction of the global layer fibers passing through the pulley to insert on the globe. It should be noted, however, that others have disputed this hypothesis (McClung et al., 2006). Demer and colleagues (Demer, 2004; Demer et al., 2000) further proposed that the outer, orbital layer can control the tension on the pulley independently of the actions of the global layer. If autonomous laminar control is present, we might expect to find anatomically distinct motoneuronal pools for those neurons innervating the outer, orbital layer, and inner global layer to facilitate this independent control. Here, using the cat lateral rectus muscle as a model, we report the results of our initial experiments to determine whether these two layers possess anatomically separate innervation.

MATERIALS AND METHODS

Experiments were performed with 12 male and female adult cats (*Felis catus domestica*). Injections of wheat germ agglutinin conjugated to horseradish peroxidase (WGA-HRP) were directed at the insertion of the medial rectus muscle and the orbital layer of the lateral rectus muscle. All procedures were conducted in accordance with National Institutes of Health guidelines for animal care and use, and were approved by the University of Mississippi Medical Center IACUC.

Surgical procedures

Surgeries were performed under sterile conditions. Animals were anesthetized with and maintained on isoflurane (1–3%). An intravenous line delivered fluid support. The animals received an injection of dexamethasone (2.5 mg/kg, IV) to minimize tissue swelling and atropine sulfate (0.05 mg/kg, IM) to control airway secretions. Vital signs were monitored throughout the procedure. With the head secured in a stereotaxic apparatus (Kopf Instruments, Tujunga, CA), the medial rectus muscle was approached by first making an incision along the brow behind the supraorbital ridge and then disinserting the orbicularis oculi muscle from the supraorbital ridge. To visualize the target muscle, connective tissue on the medial side of the orbit was bluntly dissected down to the sclera. A muscle hook was positioned along the medial surface of the globe to draw forward the medial rectus insertion, extorting the eye. A 10- μ l Hamilton syringe with a beveled needle was used to inject 1–3.5 μ l 2% WGA-HRP in dH₂O (Sigma, St. Louis, MO) into the insertion. The insertion of the orbicularis oculi muscle was then reattached, and the skin incision was closed with suture.

Monkeys have a completely enclosed bony orbit, but the orbit of the cat is only partially encased in bone. We took advantage of this feature to approach the lateral rectus muscle from the orbital side. An incision was made parallel and medial to the zygomatic process. The temporalis muscle was cut along its medial edge where it inserts into the frontal bone and its anterior edge where it inserts on the posterior side of the supraorbital ridge. It was then retracted posteriorly and held in position, revealing the temporal fossa and, inferiorly, granting access to the lateral aspect of the orbit. A small portion of the medial edge of this muscle and the underlying connective tissue were then removed to allow better visualization of the body of the lateral rectus muscle. The target muscle was dissected free of the globe to reveal its dorsal and lateral surfaces. After the muscle was isolated, a length of suture was placed around the muscle and drawn forward, causing the muscles to become taut. This facilitated driving the injection needle into the muscle. A 5.0- μ l Hamilton syringe with a glass micropipette tip affixed to the end of the needle was placed on a micromanipulator (Narishige International USA, East Meadow, NY) attached to a post. The needle was angled parallel with the outer, orbital layer of muscle. The micropipette tip was then driven from above down into the muscle as close to the lateral surface as possible, targeting the outer, orbital layer. Next, 0.1–0.2 μ l of 2% WGA-HRP was pressure injected into the muscle and we then waited 5 minutes before pulling the micropipette tip out. The temporalis muscle was stabilized with suture, and the incisions were closed. Sensorcaine (0.5–1.0 ml) was then administered locally at both incision sites. Buprenex (0.01 mg/kg, IM) was given as a postoperative analgesic.

Animals survived for ~48 hours to allow for retrograde transport of the tracer to the motoneuron somata. At this point, they were deeply anesthetized with sodium pentobarbital (50 mg/kg, IP) and were then subjected to a transcardial perfusion, first with 1.0 liter of 0.1 M PBS (pH 7.2), followed immediately by 2.0 liters of 1% para-formaldehyde and 1.25% glutaraldehyde fixative in 0.1 M phosphate buffer (PB; pH 7.2). Brains were blocked in the frontal stereotaxic plane and were postfixed in the same fixative solution for 1–2 hours before being stored in 0.1 M PB (pH 7.2) at 4 °C overnight. With a vibratome (Leica, Nussloch, Germany), 100- μ m-thick frontal sections through the brainstem were obtained

and stored in PB. Eye muscles were individually isolated and dissected out. They were allowed to postfix for 1–2 hours before being stored in 0.1 M PB (pH 7.2) at 4 °C overnight. Muscles were then transferred to a 1:1 0.1 M PB (pH 7.2): cryomatrix (Thermo Fisher Scientific, Kalamazoo, MI) for a minimum of 2 days before being stored in 100% cryomatrix for a minimum of 1 week before sectioning. Initially, sections were cut on a cryostat in different planes to explore which was most effective. We found that, to visualize the insertion injections best, it was preferable to section the medial rectus longitudinally from the outer, orbital-layer side toward the inner, global-layer side. In contrast, the lateral rectus was cut into either coronal sections or longitudinal sections because both allowed for visualization of the two layers. Muscle sections were mounted onto microscope slides and allowed to dehydrate for at least one night.

Histochemical procedures for WGA-HRP visualization

A one-in-three series of brainstem sections was pre-incubated in 0.1 M PB (pH 6.0) containing 0.25% ammonium molybdate and 0.005% tetramethylbenzidine (TMB). Next, 0.3% hydrogen peroxide was added to drive the reaction, producing a blue reaction product localized to the cell cytoplasm. Sections were incubated overnight at 4 °C to enhance the reaction, followed by stabilization in a 5% ammonium molybdate solution in 0.1 M PB (pH 6.0; for details see Barnerssoi and May, 2015). Sections were then rinsed and mounted on gelatinized slides and air dried. Sections were counter-stained with cresyl violet, dehydrated in a graded series of ethanols, cleared, and coverslipped. Eye muscle sections were rehydrated in 0.1 M PB (pH 6.0) and reacted on the slides with the aforementioned TMB protocol.

Statistical analysis

Areas of interest were drawn, and labeled motoneurons were charted with a Nikon Eclipse 80i microscope (Nikon Instruments, Melville, NY) equipped with a drawing tube. Crossed polarizers were used to reveal the reaction product better. Drawings were scanned and digitized and, in some cases, traced in vectored drawing software (CorelDraw; Corel Systems, Ottawa, Ontario, Canada). Labeled cells were photographed with a Nikon Eclipse E600 microscope equipped with a motorized stage and a 1.5-megapixel Nikon DS-Ri1 high-resolution digital camera and NIS Elements. This system has the capacity to photograph and fuse multiple z-axis focal planes. Photomicrographs were edited in Photoshop 12.1 (RRID:SciRes_000161; Adobe Systems, San Jose, CA) to match the appearance of the original material as closely as possible. Morphometric measures were conducted on live images with NIS Elements (Nikon). The dimensions measured included the area, diameter, perimeter, and shortest and longest axis lengths (minimum feret and maximum feret, respectively) of the cell body, excluding dendrites. They were obtained at $\times 40$ magnification. Measurements were stored in an Excel spreadsheet (Microsoft, Redmond, WA). Statistical analysis was performed offline in Systat 13.

RESULTS

MIF motoneuron distribution pattern and morphology

A spectrum of muscle injections was found that varied with respect to the extent of the injection site. Figure 1 illustrates two example injections of WGA-HRP placed into the medial rectus muscles of cats. The first illustrated injection (Fig. 1A) is an example in which the tracer was confined to the myotendinous junction. Thus, it appears to be well positioned to reveal the location of MIF motoneurons within III. In the second example, tracer spread beyond the myotendinous junction to include a portion of the medial rectus muscle belly (Fig. 1B). The belly of an extraocular muscle contains axons supplying both SIFs and MIFs. Consequently, larger injections aimed at the distal muscle were assumed to label both the MIF and, to varying degrees, the SIF motoneuronal pools.

Figure 2 shows four representative cases arranged in order of increasing number of retrogradely labeled medial rectus motoneurons. For each case, sections are presented from rostral to caudal. In Figure 2, medial rectus case 1 (MR case 1) reveals the distribution of retrogradely labeled MIF medial rectus motoneurons from the case with the fewest labeled cells (injection site illustrated in Fig. 1A). In this case, most of the labeled motoneurons (dots) were located along the ventrolateral edge of III where it meets the medial longitudinal fasciculus (MLF; MR case 1B–E). In fact, the labeled population often extended among the fascicles of the MLF (MR case 1B–G). Labeled cells were also seen at the rostral pole of the nucleus (MR case 1A), where they extended along its rostral surface. In MR case 2A–G, the pattern of retrogradely labeled medial rectus neurons was akin to that observed in MR case 1 but revealed an increase in the number of labeled motoneurons found along the ventrolateral edge. In addition, a few labeled cells were observed more dorsally within III (MR case 2B–E). MR case 3 was similar to the two previous cases, but even more cells were retrogradely labeled. Labeled motoneurons were again concentrated along the ventrolateral edge of the nucleus. Additionally, this injection labeled more motoneurons dorsally and dorsomedially in III (MR case 3B–E). MR case 3 had a greater number of labeled motoneurons within and ventral to the MLF compared with MR case 2 (MR case 3E–G). MR case 4 shows the distribution of labeled medial rectus motoneurons after the injection that produced the greatest number of retrogradely labeled motoneurons across all cases. The distribution pattern of labeled motoneurons along the ventral and ventrolateral edge is similar to the pattern observed in MR cases 2 and 3. Unlike the previous cases, this injection produced more cells in the dorsal group than in the ventral group (MR case 4A–G). The two populations of motoneurons appear to be largely separate motoneuronal pools. The distribution in MR case 4 is very similar to that seen with the injection site illustrated in Figure 1B (MR case 5), although MR case 5 had fewer cells labeled. The presence of the dorsal group of motoneurons appears to track with larger numbers of labeled cells (Fig. 2) and injections that extend farther from the insertion, suggesting that MIF motoneurons were located in the ventral group and that SIF motoneurons were located in the dorsal group. This pattern of labeling was still seen in cases in which there was no lateral rectus muscle injection, so we do not believe that the lateral rectus injections spread into other muscles to provide the pattern labeling shown here.

The differences and similarities in labeling patterns among cases can also be appreciated in the photomicrographs shown in Figure 3. Figure 3A, which is from MR case 1 with the smallest injection (Figs. 1A, 2), shows that a small number of weakly labeled cells (white arrow) hug the lateral edge of the nucleus, where it abuts the MLF. Figure 3B shows a photomicrograph of a section from MR case 3 (Fig. 2). The white arrows indicate ventrolaterally labeled medial rectus motoneurons, some of which lie among the fascicles of the MLF, and the yellow arrow indicates dorsomedially labeled motoneurons. Figure 3C shows a photomicrograph of the section from MR case 4 (Fig. 2) in which the most cells were labeled. Again, the white arrow indicates ventrolaterally located, labeled medial rectus motoneurons, some of which lie within the borders of the MLF, and the yellow arrow indicates dorsomedially labeled motoneurons.

The presumed MIF motoneurons located along the ventrolateral edge of the III often appear smaller compared with presumed SIF motoneurons located dorsal and dorsomedially within the nucleus (Fig. 3B). This is better illustrated by another, larger injection site in which the cells were more completely labeled (Fig. 3D). The largest labeled cells were located dorsally (Fig. 3E), more ventrally located cells in III were somewhat smaller (Fig. 3F), and those found among the fascicles of the MLF were smaller still (Fig. 3G). Based on this observation, the somatic area values for all observable labeled medial rectus motoneurons in all medial rectus cases were calculated. Figure 4A shows the distribution of the soma area values for the dorsal group (dashed line) and the ventral group (solid line). The median soma area for the dorsally located group was $569.99 \mu\text{m}^2$, with a range of $147.68\text{--}1,519.19 \mu\text{m}^2$, whereas the ventrally located cell group had a median value of $322.196 \mu\text{m}^2$, with a range of $92.62\text{--}1,115.32 \mu\text{m}^2$. Application of the Mann-Whitney test to the median soma area values revealed a significant difference in the distributions of the two groups (Mann-Whitney test, $U_1 = 285,544.50$, $\chi^2 = 347.341$, $P < 0.001$). Differences were also observed in the other measures (e.g., perimeter; see Table 1). However, because these generally track with the somatic area, they are not discussed in detail here.

Given the observed size differences, we compared the findings from individual cases. To assess whether there were differences in the distributions of retrogradely labeled motoneuronal soma sizes, it was essential first to normalize the values relative to the total number of labeled cells in the case because this number was quite variable. To do this, raw measures for soma area (micrometers squared) were separated into $100\text{-}\mu\text{m}^2$ bins. Total counts for each bin were divided by the total number of cells in the case and then plotted to gain an idea of possible differences in size distribution (Fig. 4B). Close inspection reveals an apparent clustering of distributions. The first cluster of cases was composed largely of cells with smaller somata (MR cases 1–3, 6; Fig. 4B). The second cluster of cases contained far more cells with larger somata in addition to neurons with smaller somata, so the distribution of labeled soma areas was pushed to the right (MR cases 4, 5, 7; Fig. 4B). The smaller, leftward cluster shown in Figure 4B is composed of cases that had primarily ventral medial rectus motoneurons labeled and shows limited labeling of dorsal neurons. Based on cell location differences (Figs. 2 and 3) paired with the realization that the cases charted in Figure 4B showed the same clustering among cases, we then collapsed the individual cases into two groups. The first group consisted of cases in which the vast majority of labeled cells was located ventrally within III (Fig. 4C, solid line). This included cases MR 1–3 from

Figure 2. The second group consisted of cases in which substantial numbers of labeled cells were located both ventrally and dorsally within III (Fig. 4C, dashed line). This group included MR case 4, illustrated in Figure 2. When the somal area was normalized, the distribution was pushed to the right (compare dashed with solid line; Fig. 4C). Median soma areas for distal insertion and proximal body injections were $301.421 \mu\text{m}^2$ with a range of $92.62\text{--}1,373.59$ and $448.442 \mu\text{m}^2$ with a range of $121.88\text{--}1,519.19$, respectively. The soma size distributions of the two groups differed significantly (Mann-Whitney $U_1 = 2,338.00$, $\chi^2 = 4.158$, $P = 0.041$). This difference occurred presumably because the cases represented by the solid line labeled primarily MIF motoneurons, whereas those represented by the dashed line included significant populations of both SIF and MIF motoneurons.

Orbital layer motoneuron distribution pattern and morphology

Extraocular muscles are composed of two distinct layers, the outer, orbital layer and the inner, global layer. To test for the existence of distribution patterns or other morphological features specific to the layer innervated, we attempted to inject just the orbital layer of the lateral rectus muscle. Two examples of these injections are shown in Figure 5. In some cases, the injection of WGA-HRP appeared to be constrained to the outer, orbital layer of the lateral rectus muscle (Fig. 5B). In other cases, tracer spread beyond the orbital layer, invading the inner, global layer of the muscle to some degree (Fig. 5A). The largest injections (not illustrated) included substantial portions of both layers, and they resulted in far more cells being labeled. Because of the narrowness of the layers, it was essential to make very small injections to attain layer specificity, and these injections, by definition, labeled only a small minority of the abducens motoneurons.

Figure 6 shows four representative cases in which the orbital layer of the lateral rectus muscle was targeted with WGA-HRP. Based on the number of labeled cells (dots), the injection sites in these cases range from small (lateral rectus [LR] case 1) to large (LR case 4), with increasing involvement of the global layer. LR case 1 plots a rostral to caudal series through the abducens nucleus from the case whose injection site is illustrated in Figure 5B. Only a handful of labeled, lateral rectus motoneurons were present after this very small injection. These orbital layer motoneurons were interspersed among numerous unlabeled abducens cells. Despite the small number of cells labeled, no obvious distribution pattern of orbital layer motoneurons is evident within the nucleus. Thus, in LR case 1, labeled cells are present both rostrally (A) and caudally (F), both dorsally (A) and ventrally (C), and both medially (D) and laterally (C). LR cases 2 and 3 (Fig. 6) display the distribution pattern of lateral rectus motoneurons from examples in which a somewhat larger number of cells was labeled. The injection site for LR case 2, which only slightly involved the global layer, is illustrated in Figure 5A. Similarly to LR case 1, labeled motoneurons in both these cases were randomly distributed among the counterstained abducens neurons, so, in LR cases 2 and 3, labeled cells were present from the rostral (A) to caudal (F) end of the nucleus and stretched across its mediolateral and dorsoventral extent (A–F). Finally, a case with large numbers of labeled cells is illustrated for comparison (LR case 4). This injection clearly involved both layers of the lateral rectus muscle and labeled many abducens motoneurons, which filled the extent of the nucleus. It is apparent from these examples that no obvious

pattern in the distribution of lateral rectus motoneurons was evident despite variation in the number of cells labeled and, presumably, the degree of global layer involvement.

The characteristics of the abducens motoneuron labeling can be appreciated in the photomicrographs shown in Figure 7. Figure 7A shows labeled cells from a large, nonlayer-specific injection such as in LR case 4, illustrated in Figure 6. The nucleus is filled with multipolar neurons. As shown in the inset, they vary in soma size. Figures 7B and 7C show examples from two cases that displayed moderate numbers of labeled abducens motoneurons (LR cases 3 and 2 from Fig. 6, respectively). The labeled cells are dispersed within the dorsoventral and mediolateral extent of the nucleus. As shown in the insets, the size of their somata varies. Figure 7D shows the pattern of labeling from a case with the smallest number of labeled cells (LR case 1; Fig. 6). As can be seen in both Figure 7C and Figure 7D, with the smallest injections, the motoneurons were also more weakly labeled. Nevertheless, there was still variation in the size of the labeled cells (insets).

The lack of compelling anatomical evidence in support of segregated distribution patterns of motoneurons based on muscle layer does not exclude the possibility that morphological differences between orbital layer and global layer abducens motoneurons might exist that were not immediately evident from visual inspection. Consequently, abducens motoneuron data were plotted as a distribution with regard to somal area for eight lateral rectus cases (Fig. 4D). The two smallest cases are excluded from Figure 4 because we were afraid that their extremely low sample size ($n = 4$ and $n = 9$) might skew the rest of the data. However, it should be noted that, despite the low sample size, the range and median of the raw data for the two excluded cases ($n = 13$, median somal area $630.983 \mu\text{m}^2$, range $181.717\text{--}915.335 \mu\text{m}^2$) had remarkable overlay with the distribution of samples from the included cases ($n = 2,125$, median somal area $705.598 \mu\text{m}^2$, range $137.538\text{--}2,475.399 \mu\text{m}^2$). As can be observed in Figure 4D, despite a massive range in the number of cells labeled, the distribution pattern is highly conserved with minimal variation across cases. Presumably, the number of labeled cells varies with the effective injection size, as does the likelihood of including portions of the global layer, so we believe that this analysis also indicates that there is no correlation between the degree of inclusion of the global layer and somal size. As a result, subsequent grouping or statistical testing was deemed unnecessary.

DISCUSSION

Based on injections of retrograde tracer into the insertion of the medial rectus muscle, it appeared that the cat had partially segregated MIF and SIF motoneuronal populations. The MIF and SIF motoneuronal populations also displayed morphological differences similar to those observed in previously described species (pigeon [Erichsen and Evinger, 1989; Erichsen et al., 2000], rat [Eberhorn et al., 2006], and monkey [Büttner-Ennever and Horn, 2002; Büttner-Ennever et al., 2001; Eberhorn et al., 2005a]). In contrast, injections of retrograde tracer into the outer, orbital layer of the lateral rectus muscle did not reveal an anatomically distinct population of motoneurons compared with full-muscle injections. These cat results argue against laminar-specific neuronal control of the extraocular muscles. The implications of these two findings are discussed below.

Technical considerations

The results from this study must be considered within the limitations of the techniques employed. Just as with all tracer experiments, the primary considerations are tracer spread and effective uptake area. We dealt with the first limitation by performing increasingly smaller injections for both experiments and by quantifying the number of centrally labeled cells. The largest injection into the cat medial rectus muscle labeled 424 neurons, whereas the smallest injection resulted in just 46 cells being labeled. Based on the fact that the distal insertion was targeted, we assumed that, when smaller numbers of cells were labeled, the injection was better localized to the insertion of the muscle and was less likely to involve uptake from SIF terminals. Consistent with these assumptions, the data produced clear statistical distinctions under these conditions. Thus, small distal injections labeled small, ventrolaterally located motoneurons almost exclusively, whereas larger injections that spread further into the muscle belly labeled more large cells and more dorsal cells. The small, ventrolateral population likely represents MIF motoneurons and the large, dorsomedial population likely represents SIF motoneurons. Specifically, this assumption led to cell size vs. injection site correlations that paralleled those seen in other species (Büttner-Ennever and Horn, 2002; Büttner-Ennever et al., 2001; Eberhorn et al., 2005a.). This assumption also supported the contention that MIF and SIF motoneurons occupy different locations within III. We were not able to obtain good staining from all the muscles in all the medial rectus cases that we analyzed, and, in some cases, spread along the epimysium of the muscle produced variable labeling centrally. A wider distribution of en plaque terminals is present in cats (Mayr et al., 1975) than in primates, making these experiments more challenging. Nevertheless, we did find cases in which an injection site was confined to the insertion, and these resulted in the labeling of small motoneurons, distributed adjacent to and within the MLF.

Because two separate pools of cells were seen after medial rectus muscle injection, spread of tracer to different extraocular muscles within the orbit was an additional concern, but we do not believe that this was an issue for the following reasons. First, the motoneuronal pools of the cat extraocular muscles are well established (Akagi, 1978; Miyazaki, 1985). If there was spread to another muscle, we would expect labeled neurons in locations outside the previously described medial rectus motoneuronal pools. Weight is added to this argument by the realization that there was a good degree of consistency in the pattern of labeled cells among individual cases. Spread to other muscles or their nerves would have produced obvious variations in this pattern.

Spread of the tracer beyond the outer, orbital layer of the lateral rectus was also a concern. An injection and subsequent diffusion of tracer results in a three-dimensional sphere or teardrop shape, in which the densest tracer is observed along the track left by the injection syringe. As a result, injections constrained to a thin layer are difficult to make. We attempted to address this constraint primarily by making the injected amounts progressively smaller. Although this was effective, it also reduced the number of cells labeled and the density of label within the cells. A second approach was attempted whereby dehydrated wafers of WGA-HRP were placed on the surface of the lateral rectus (data not shown) in the hope that the tracer would diffuse through the epimysium and into the outer layer. However, no labeled

motoneurons were found within the abducens or other extraocular motor nuclei in these cases. This finding may speak to the propensity of WGA-HRP to spread within the orbit to access other muscles.

MIF and SIF motoneuron pools

The present study is the first to demonstrate a specific link between motoneuronal pools and muscle fiber innervation patterns in the cat. The cat appears to share some organizational features with the rat, but other characteristics are more reflective of the primate's pattern of extraocular muscle innervation. In rats the MIF and SIF motoneurons pools generally overlap within the extraocular nuclei (Eberhorn et al., 2006), whereas in primates SIF motoneurons lie within the nuclei, and MIF motoneurons lie at the periphery of each nucleus (Büttner-Ennever and Horn, 2002; Büttner-Ennever et al., 2001; Eberhorn et al., 2005a). More specifically, in the case of medial rectus motoneurons, rat MIF and SIF motoneurons are intermixed in a single medial rectus motoneuronal pool inside III (Eberhorn et al., 2006). Monkey medial rectus SIF motoneurons are found in the ventrolaterally located A-group and dorsocaudally located B-group within III, but the MIF motoneurons for this muscle lie outside the dorsomedial edge of III in C-group (Fig. 8). There are also separate populations of smaller and larger medial rectus motoneurons in the pigeon (Erichsen and Evinger, 1989; Erichsen et al., 2000). The present results demonstrate that in the cat, just as in the rat, both MIF and SIF motoneurons lie mainly within the borders of III. However, we have presented evidence that the cat differs from the rat in that there is at least partial segregation within III of the MIF and SIF populations supplying the medial rectus muscle. The presence of this segregation parallels the segregation found in the monkey, but the specific pattern of that segregation is different.

The finding of dorsal and ventral populations after retrograde labeling of the cat medial rectus motoneurons is not novel; these populations have been observed many times (Akagi, 1978; Gacek, 1974; Miyazaki, 1985; Naito et al., 1974; Spencer and Porter, 1981; Tarlov and Tarlov, 1971). Miyazaki (1985) discussed and subsequently dismissed the hypothesis that the two subgroups were related to fiber type in the cat and instead suggested that the arrangement of medial rectus motoneurons in the cat resembles that of the monkey A- and B-groups because there is a ventrolateral group adjacent to the MLF (such as the primate A-group) and a second, more dorsally located population (such as the primate B-group). Other authors have put forth the possibility that the dorsal population may be the homologue to the primate C-group, based on its location (e.g., Tang et al., 2015). However, our results for the cat are not in agreement with either proposal. Instead, they show that the ventral population of motoneurons appears to be composed primarily of MIF motoneurons, and the SIF population is located primarily dorsally. Thus, the distribution of these two populations into ventral and dorsal subgroups in this species is not equivalent to the primate A- and B-groups. In primates, a small number of labeled MIF motoneurons can be observed ventrolaterally within III after smaller distal tracer injections of the medial rectus muscle (see, e.g., Fig. 7B,C of Büttner-Ennever et al., 2001).

Miyazaki (1985) reported that this ventral population, which extends into the medial longitudinal fasciculus, constituted about 20% of the motoneurons. If this group is mainly

MIF motoneurons, then the numbers are in good agreement with Eberhorn and colleagues' (2006) finding that MIF motoneurons constitute 15–22% of the total motoneuronal population in the rat. Similarly, the orbital layers of rectus and oblique muscles reportedly consist of a ratio of ~80:20 SIFs:MIFs, whereas the global layer consists of a ratio of ~90:10 SIFs:MIFs (human [Wasicky et al., 2000], rat [Mayr, 1971]), although we do not know the size of the MIF or the SIF motor units. Together with the present results, this indicates that MIFs and their motoneurons constitute a minor, but nevertheless significant, component of the cat medial rectus muscle and its innervation. In this light, the fact that we still do not know the firing characteristics of MIF motoneurons or the functional role of MIFs remains a noteworthy gap in our knowledge of oculomotor system function.

We have shown that the morphological measures that factor for location within the nucleus reveal size differences between the two feline medial rectus motoneuron populations (Fig. 4). We would argue, in the context of the injection site locations, that this indicates that, in the cat, MIF motoneurons are significantly smaller than SIF motoneurons. It should be noted, however, that our analysis cannot distinguish whether the ventrolateral group consists of purely MIF motoneurons. It is likely that some SIF motoneurons are present, so the pool is somewhat intermixed just as in the rat, although clearly trending toward the nearly complete separation of the monkey. This difference in size is a shared feature that has been observed in both rats and monkeys (rats [Eberhorn et al., 2006], monkeys [Büttner-Ennever and Horn, 2002; Büttner-Ennever et al., 2001; Eberhorn et al., 2005a]). Thus, it is likely related to morphological differences that undergird the physiological characteristics and functions of these two motoneuron types. In fact, when Nelson and colleagues (1986) looked at the electrophysiology of the cat superior oblique muscle, they found that fibers showing graded potentials (presumed MIFs) were supplied by smaller, more slowly conducting axons than the twitch fibers (presumed SIFs).

One of the features of the MIFs in the global layer is the presence of palisade endings where they insert into the tendon. Although these have long been considered to be proprioceptors (Billig et al., 1997; Büttner-Ennever and Horn, 2002; Donaldson, 2000; Eberhorn et al., 2005c; Lienbacher and Horn, 2012; Lienbacher et al., 2011a,b; Ruskell et al., 2005; Steinbach, 1987; Weir et al., 2000), the axons giving rise to the palisade endings also display motor features (Eberhorn et al., 2005b; Konakci et al., 2005a,b; Rungaldier et al., 2009). Recent evidence from cats and monkeys indicates that these palisade endings originate from MIF motoneurons, and they have been reported to be extensions of the axon supplying the MIF (Büttner-Ennever et al., 2001; Konakci et al., 2005a,b; Zimmermann et al., 2011, 2013). This has led to argument over whether palisade endings truly represent extraocular muscle proprioceptors (Rao and Prevosto, 2013; Zimmermann et al., 2011, 2013). Anatomical support for the palisades serving a proprioceptive role was recently provided by Lienbacher and colleagues (2011a,b), who compared monkey cases with injections constrained to the tendinous insertion (containing the palisade endings) vs. injections into the distal muscle (containing MIF terminations). In the former case, the label was found in round, medium-sized somata located near the preganglionic Edinger-Westphal (EWpg) nucleus, whereas, in the latter case, the labeled somata were smaller, multipolar neurons located immediately peripheral to III. In the current study, we did not see any great difference in morphology or distribution within the ventrolateral group of presumed MIF motoneurons when the injection

was largely in the insertion (see Fig. 1) or spread beyond it, except that there were greater numbers of cells within the MLF in the latter case. However, the number of cases is too small to make a definitive statement about this trend.

Global and orbital layer innervation

Based on the results reported here, there appears to be little substantiating evidence for anatomical specializations in the organization of lateral rectus motoneurons that supply the outer, orbital layer of the lateral rectus muscle. These distribution results suggest a lack of anatomically distinct motoneuronal pools innervating the two layers. This lack of distributional segregation argues against independent motoneuronal control of the global and orbital muscle layers, although differences in the peripheral organization of the layers would still produce different effects. The most obvious difference is the location of each layer's insertion. Given that the orbital layer inserts onto the tendinous pulley, it will have a different response and action than the global layer, which inserts on the sclera, even if it receives identical neuronal signals. We also did not see any evidence for morphological differences suggesting that the two layers received input from different types of motoneurons. This lack of morphological difference is perhaps not surprising in light of the fact that both layers contain MIFs and SIFs and that the other experiment reported here indicates that a smaller soma correlates with motoneurons supplying MIFs.

Our findings do not specifically address the active pulley hypothesis (Demer, 2002) *per se*; it is still possible that muscle-layer-specific inputs to the abducens nucleus could differentiate motoneurons within this mixed population based on molecular signals. In addition to receiving different inputs, they could potentially have different membrane channel characteristics. These factors could result in orbital and global layer motoneurons having different firing properties. However, there is little evidence from recordings made from extraocular motoneurons that physiologically discrete subpopulations exist. Instead, these motoneurons have been reported to display a continuous spectrum of physiological characteristics, such as rate constants and thresholds linked to the eye's position within the orbit (Delgado-Garcia et al., 1986; Keller, 1973; Keller and Robinson, 1972; Mays and Porter, 1984; Robinson, 1970; Robinson and Keller, 1972). To the best of our knowledge, Henn and Cohen (1972) are the only authors who argue against all extraocular motor neurons sharing the same patterns of activity. Alternatively, it remains possible that differences in muscle physiology could result in differences in function between the two layers, even if they receive essentially the same signal from their motoneurons. The fact that the spectrum of fiber types differs between the layers (Spencer and Porter, 1988) supports this contention. One final point worth noting is that the horizontal rectus muscles seem to be compartmentally innervated, with superior and inferior divisions (Demer and Clark, 2014; Peng et al., 2010; Shin et al., 2015). Unfortunately, the experiments performed in the present study were not designed to address this issue.

The orbital layer contains MIFs; however, orbital layer MIFs can receive an additional en plaque ending in the middle of the fiber (Jacoby et al., 1989). The motoneurons supplying these MIFs were presumably labeled by our orbital layer injections. Indeed, the lower range of soma sizes seen after abducens injections matches the range of soma sizes for medial

rectus MIF motoneurons described here. We did not see any evidence that the orbital layer MIF abducens motoneurons differed from global layer MIF medial rectus motoneurons. However, we did not determine the distribution of either orbital or global layer abducens MIF motoneurons. Additional experiments must be conducted in which MIFs from each of the layers of an individual muscle are independently labeled to characterize definitively the motoneurons supplying the orbital layer.

Functional rationale for species differences

Close examination of the morphology and ultrastructure of C-group medial rectus MIF motoneurons in the monkey has revealed that their dendrites extend into the supraoculomotor area (SOA) and the EWpg, and that most synaptic contacts are on these dendrites (Erichsen et al., 2014; Tang et al., 2015). The SOA contains vergence neurons, whereas the EWpg contains neurons driving lens accommodation and pupillary constriction (Das, 2011, 2012; Gamlin et al., 1994; Judge and Cumming, 1986; Mays, 1984; Mays et al., 1986; Fig. 8B). Consequently, Erichsen and colleagues (2014) concluded that the C-group dendrites likely receive the same premotor synaptic inputs as the EWpg neurons and may therefore serve the accommodative vergence drive as part of the near triad. A similar argument has been made for the pigeon, in which the small cell pool of the medial rectus lies closer to EW and extends dendrites into the this nucleus (Erichsen and Evinger, 1989; Erichsen et al., 2000).

At the outset of these experiments, we hypothesized that cats and monkeys would display similar MIF and SIF motoneuron pool organizations because of the similarities in their visuomotor behavioral repertoires. On the face of it, it appears that the results failed to confirm this initial hypothesis. However, it may be that there is a functional organization of the cell groups related to the near triad, but it must take into account the differences in the organization of the relevant populations between cats and monkeys. As schematized in Figure 8A, cats and monkeys show a number of anatomical differences in the locations of known cellular populations within III and lying dorsal to III in the SOA (Kozicz et al., 2011). Note that the cholinergic EWpg population supplying the ciliary ganglion can be distinguished from the peptidergic centrally projecting population of Edinger-Westphal (EWcp) neurons in both species, and that the cytoarchitectonically defined EW nucleus has different contents in cats (Fig. 8B,C) than in monkeys (Fig. 8D,E). In primates, EWpg neurons form a tight cluster within the anteromedian nucleus (AM) found immediately rostral to III (Fig. 8D) and within the cytoarchitectonically defined EW nucleus found dorsal to III (Fig. 8E). The EWcp neurons are diffusely arranged within the SOA, including the AM, but avoid the cytoarchitectonically defined EW nucleus. In contrast, the cat cytoarchitectonically defined EW nucleus contains mainly EWcp neurons (Fig. 8C). It is the EWpg neurons that are distributed diffusely within the SOA and the AM (Fig. 8B,C). They also extend ventral to the MLF (Fig. 8C). The MIF motoneurons in the cat are present at the rostral pole of III and along its ventrolateral edge. In view of this organization, one interpretation may be that, just as with the primate medial rectus MIF motoneurons, the cat MIF motoneurons do maintain a close relationship with EWpg neurons to share afferent inputs. Thus, because the cat EWpg cells are located mainly ventral and rostral to III, not dorsal to III, the MIF motoneurons are found mainly along the rostral and ventrolateral

border of III and in the MLF. In fact, Erichsen and May (2002) have suggested that the cat preganglionic motoneurons controlling lens accommodation are found mainly in the adjacent area beneath the MLF, so this increases the likelihood that cat MIF motoneurons are seeking to share lens accommodation inputs. Certainly, the results of this study in the context of previous work suggest that the organization and function of the SOA differs between species.

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Figure 1.

Examples of injection sites in medial rectus muscles of two different cases. The medial rectus muscles were cut longitudinally, and the section illustrated is located at the point at which the most label is evident. **A:** In MR case 1, the WGA-HRP was found primarily in and adjacent to the insertion of the muscle. **B:** In MR case 5, the WGA-HRP injection spread beneath the epimysium along the edge of the medial rectus belly. [Color figure can be viewed at wileyonlinelibrary.com]

Figure 2.

Chart of retrogradely labeled motoneurons (dots) distributed throughout the rostral–caudal extent of the left cat III (A–G) following small, medium, and large injections of WGA-HRP into the left, distal medial rectus muscle. MR case 1: A small injection resulted in labeling of a population of motoneurons located along the ventrolateral edge of III. Labeled cells also were found within the borders of the adjacent MLF. MR cases 2, 3: Larger injections produced an additional motoneuronal population located dorsomedially within the nucleus. MR case 4: A large muscle injection revealed a more complete distribution of labeled medial rectus motoneurons within III. This also resulted in more labeled cells located ventral to the MLF (E–G). Scale bar = 0.5 mm. [Color figure can be viewed at wileyonlinelibrary.com]

Figure 3.

Photomicrographs showing labeled medial rectus motoneurons within III and MLF. Illumination with crossed polarizers reveals cells located ventrolaterally in III and in the adjacent MLF (white arrows) and cells located dorsomedially within III (yellow arrows). **A:** Labeled medial rectus motoneurons present ventrally within III resulting from an injection of WGA-HRP that was constrained to the insertion of the muscle (from MR case 1; Fig. 2). **B:** Labeled medial rectus motoneurons from an injection with limited spread beyond of the insertion, labeling the ventral motoneuron population as well as some dorsal cells (from MR case 3; Fig. 2). **C:** Labeled medial rectus motoneurons from injection with considerable spread beyond of the insertion, labeling both the ventral and the dorsal motoneuron populations (from MR case 4; Fig. 2). **D:** Low-magnification photomicrograph from an additional case with a large injection with well-labeled cells. Outlined areas show the locations of E–G. **E:** The somata of medial rectus motoneurons located dorsally in III are quite large. The somata of medial rectus motoneurons located ventrally (**F**) in III and in the MLF (**G**) are smaller. Scale bars = 100 μm in D (applies to A–D; 100 μm in G (applies to E–G)).

Figure 4.

Morphometric characterization of labeled medial and lateral rectus motoneurons. **A:** Cell size distribution between dorsal and ventral populations of medial rectus motoneurons in III. Cases were collapsed, and cell location was then factored across cases. It is important to note that the number of labeled cells for the ventral population appears larger because there were many more cases in which the injection was confined to the insertion of the medial rectus, whereas there were fewer cases in which the tracer labeled all motoneurons projecting to the medial rectus. **B:** Distribution of labeled medial rectus motoneuronal cell body area as a percentage of the norm across all cases. The cell numbers for each case are normalized for different sample sizes. There is a clear bimodal distribution with respect to somal area. As the injection size increases, the distribution has a relatively abrupt rightward shift resulting from an increased ratio of larger labeled motoneurons (Fig. 2 cases are indicated). **C:** After visual inspection of the labeled medial rectus motoneuron distributions in B, cases were collapsed into cases skewed left vs. cases skewed right. **D:** Morphometric analysis of labeled cells in the abducens nucleus after WGA-HRP injections targeting the orbital layer of the lateral rectus muscle. There is no obvious difference between the curves.

Despite a large range of injection sizes and presumed spread beyond the orbital layer, based on the number of cells labeled, there is little evidence for differences in somatic area.

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Figure 5.

Examples of tracer injections into the outer orbital layer of the lateral rectus muscle. **A:** Example of a lateral rectus muscle (LR case 2) cut transversely such that both the orbital and the global layers can be observed at different points along the muscle from the proximal to the distal ends. **Inset** at bottom shows the approximate location from which the sections (1–3) were taken, section 3 being closest to the distal end. Stippling indicates locations where tracer could be observed within the muscle. In this example, the tracer injection is located primarily in the outer, orbital layer. **B:** Second example of a lateral rectus muscle (LR case 1) cut horizontally, such that the layers could be observed within the same section from the distal to the proximal ends. **Inset** at bottom right shows a transverse view indicating the approximate location of the drawn sections (1–3). In this case, the very small injection site of the injection was entirely constrained to the upper edge of the outer, orbital layer of the muscle. [Color figure can be viewed at wileyonlinelibrary.com]

Figure 6.

Chart of labeled lateral rectus motoneurons distributed through the rostral (A) to caudal (F) extent of the cat abducens nucleus in four cases in which orbital layer injections of WGA-HRP were attempted. Examples are organized from smallest (LR case 1) to largest (LR case 4) number of cells labeled. LR case 1: Labeled motoneurons (dots) were sparse and randomly distributed throughout. LR cases 2, 3: Medium-sized populations. The resultant distribution of labeled motoneurons within the abducens nucleus included the entire nucleus. LR case 4: Results from a lateral rectus muscle injection in which most motoneurons in the nucleus were labeled. VI, abducens nucleus; VIn, abducens nerve; VIIg, genu of the facial nerve; VIIIn, facial nerve. [Color figure can be viewed at wileyonlinelibrary.com]

Figure 7.

Photomicrographs showing WGA-HRP-labeled lateral rectus motoneurons within the abducens nucleus (VI) from four example cases. Crossed polarizers were used to reveal the reaction product in the motoneurons. **A:** Labeling of motoneurons in VI after an injection (LR case 4) that spread through the whole lateral rectus muscle. This resulted in labeled motoneurons below the genu of the facial nerve (VIIIn) distributed throughout VI. These motoneurons varied in size (see **inset**). **B:** Labeled motoneurons from a case (LR case 3) with less spread of tracer into the inner, global layer of the lateral rectus muscle resulted in fewer labeled motoneurons; however, they were still distributed throughout the abducens nucleus, and the sizes were still highly variable (see **inset**). **C,D:** Small tracer injections (LR cases 2,1) confined to the outer, orbital layer resulted in far fewer labeled cells. These were present randomly through the abducens nucleus. Furthermore, the labeled cells were variable in terms of somal area. Scale bars = 200 μm in D (applies to A–D); 50 μm in A inset; 50 μm in D insets (applies to B–D insets).

Figure 8.

Perioculomotor and medial rectus cell populations. **A:** Targets of the populations. **B–E:** Schematic comparison of known perioculomotor cell distributions between the cat and the monkey. **B,D:** Distribution in the AM of cats and monkeys. AM contains a mix of centrally projecting peptidergic neurons (dots) and peripherally projecting, preganglionic neurons (stars). The organization at the level of III is shown in **C,E**. The cat (**C**) has a cytoarchitectonically defined EW nucleus, which is composed predominantly of EWcp cells. The EWpg neurons are distributed diffusely dorsal to III in the SOA and ventral to the MLF. In contrast, the monkey (**E**) preganglionic neurons lie within the cytoarchitectonically defined EWpg nucleus, whereas the EWcp population is diffusely distributed throughout the SOA. Medial rectus MIF motoneurons (squares) and SIF motoneurons (pentagons) have species-specific locations as well. In the cat, medial rectus MIF motoneurons lie ventrolaterally within III adjacent to the MLF and among its fascicles (**C**). In the monkey, these same MIF motoneurons lie dorsal to III adjacent to the EWpg (**E**). In the cat, medial rectus SIF motoneurons lie dorsomedially within III (**C**). In the monkey, the medial rectus muscle is innervated by two anatomically distinct SIF motoneuron pools: A group, which lies ventrolaterally, and B group, which lies dorsolaterally, within III (**E**).

TABLE 1
Comparison of Morphological Measures Between Ventral and Dorsal Medial Rectus Cell Populations

	Ventral population (n = 853)					Dorsal population (n = 406)					
	Median	Mean	Range (minimum–maximum)	95% CI of arithmetic mean (LCL–UCL) ²		SD	Median	Mean	Range (minimum–maximum)	95% CI of arithmetic mean (LCL–UCL)	
Area (µm ²)	322.20	347.26	92.62–1,115.32	337.11–357.40		150.97	569.99	614.47	147.68–1,519.19	589.05–639.88	
Equator diameter (µm)	20.25	20.56	10.86–37.68	20.27–20.86		4.39	26.94	27.36	13.71–43.98	26.80–27.93	
Perimeter (µm)	71.10	72.25	35.65–127.15	71.22–73.27		15.23	90.52	91.35	45.50–141.08	89.53–93.18	
Minimum feret	15.22	15.90	6.56–32.58	15.61–16.19		4.31	23.25	23.47	11.13–40.13	22.89–24.05	
Maximum feret	28.68	29.11	13.02–54.21	28.66–29.56		6.68	33.72	34.29	16.63–58.02	33.59–34.98	
Average feret (diameter)	21.95	22.51	9.79–43.40	22.14–22.88		5.50	28.49	28.88	13.88–49.08	28.24–29.52	
										260.51	
										5.81	
										18.71	
										5.99	
										7.13	
										6.56	

¹ Lower Confidence Limit.

² Upper Confidence Limit.