Investigation of Selective Innervation of Extraocular Muscle Compartments

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Purpose. Recent magnetic resonance imaging studies have suggested that extraocular muscles (EOM) are further divided into transverse compartments that behave differentially and often unexpectedly during eye movements. Selective innervation of EOM compartments may explain the observation that certain horizontal recti compartments contribute to specific vertical eye movements and that some cyclovertical EOM compartments do not contribute to vertical vergence. We investigated the discharge characteristics of extraocular motoneurons during these eye movement tasks where EOM compartments behaved differentially for evidence of selective innervation.

Methods. We recorded from all six extraocular motoneuron populations in the abducens, oculomotor, and trochlear nuclei as two non-human primates performed vertical vergence and vertical smooth-pursuit. The relationship between motoneuron firing rate, horizontal and vertical eye parameters of the innervated eye during each task was determined using multiple linear regression.

Results. All 26 medial rectus motoneurons recorded showed no significant modulation during vertical smooth-pursuit and vertical vergence. Twenty-eight of 30 abducens motoneurons showed no significant modulation during vertical vergence, and all 30 cells did not modulate during vertical smooth-pursuit. For the cyclovertical motoneurons, 147 of the 149 cells (44/46 inferior rectus, 27/27 superior oblique, 41/41 superior rectus and 35/35 inferior oblique) modulated significantly during vertical vergence.

Conclusions. Extraocular motoneuron activity during vertical vergence and vertical smooth-pursuit does not support the theory that EOM compartments are selectively innervated. The observed differential behavior of EOM compartments is likely not driven by oculomotor control and could be due to passive change in EOM cross-sectional area.

Keywords: extraocular muscle, oculomotor mechanics, vertical vergence, motoneurons, innervation

The extraocular muscles (EOMs) have two distinct layers: a global layer that inserts on the eyeball and rotates the globe and an orbital layer that inserts on the pulleys to determine the path and pulling direction of the EOM.[1,2] In addition to these longitudinal layers, a series of recent anatomical, biomechanical and magnetic resonance imaging (MRI) studies by Demer and colleagues have suggested that the EOMs have transverse compartments that could effectively function as separate, independent muscles. These observations suggested that the horizontal recti have superior and inferior compartments, whereas the cyclovertical EOMs have medial and lateral compartments. It has been shown that human EOMs are composed of parallel fiber bundles with minimal lateral interconnections, and that their tendons are wide and insert across a broad extent of the sclera.[3,4] This could theoretically allow different amounts of force to be applied to the different scleral locations. Studies by Shin et al.[5,6] in excised bovine EOMs have suggested that their compartments are indeed mechanically independent, so that a force applied to one compartment is limited to only that compartment. Also, most motor nerves from the abducens, oculomotor, and trochlear nuclei, which supply the EOMs divide into two branches before innervating approximately equal, non-overlapping muscle regions in humans and monkeys.[7–9] These findings create the possibility for the EOM regions/compartments to be selectively innervated, and so, receive different motor commands from separate motoneuron pools, a feature found in certain human skeletal muscles.[10–13] However, whether such separate motoneuron pools exist is as yet unknown and thus was the subject of this investigation.

Functional implications of compartmentalization for oculomotor control are quite extensive. A hypothesis that emerged from possible differential compartmental behavior is that even the horizontal recti; medial rectus muscle (MR) and lateral rectus muscle (LR) could contribute toward or generate vertical and torsional eye movements. For example, selective contraction of the superior compartments of the MR and LR could generate small upward eye movements with a torsional component. Also, selective contraction of specific cyclovertical EOM compartments during vertical eye movements may imply that the other compartments of these EOM are not contributing to those vertical eye movements. Demer and Clark[14] have investigated some

of these functional effects by using MRI to observe changes in the posterior partial volumes of EOMs as a proxy for direct measurement of EOM contractility. These MRI studies describe compartmental changes in certain eye movement tasks, such as vertical vergence (disjunctive movement of the eyes along the vertical plane to compensate for vertical retinal disparity), ocular counter-rolling,[15] horizontal vergence,[16] and vertical gaze changes.[17]

What then is the mechanism behind such compartmental behavior of EOMs? This behavior combined with the observation of apparent branching of motor nerves to innervate different parts of the EOM, has led to the suggestion that compartmentalization could be under neural oculomotor control. If the observed differential EOM compartmental behavior is indeed programed from within the brain, then it should be driven by central oculomotor commands that are reflected within extraocular motoneuron activity during eye movements where the EOM compartments behaved differently.

We sought to test some of these predictions by recording from motoneurons in the abducens, oculomotor, and trochlear nuclei during specific eye movements (vertical vergence and changes in vertical gaze) that show apparent differential compartmental behavior within EOMs.[14,17] Our overall hypothesis was that selective innervation of EOM compartments would manifest as a subpopulation of horizontal motoneurons that are modulated during vertical eye movements and a subpopulation of cyclovertical motoneurons that do not modulate during vertical vergence. A more specific set of predictions emanating from the MRI compartmentalization observations are the following. First, significant contraction of the inferior LR compartment was observed in the eye that infraducted during asymmetric vertical vergence. A prediction from this behavior is that a subpopulation of lateral rectus motoneurons (LRMNs) supplying the inferior compartment will have a significant vertical position sensitivity during vertical vergence. Second, Demer et al.[17] described significant contraction of the superior MR compartment during upward gaze changes, which would predict the presence of medial rectus motoneurons (MRMNs) with a significant vertical position sensitivity. Third, during asymmetric vertical vergence, only the medial inferior rectus (IR) compartment of the eye that moved downward contracted. A prediction from this behavior is that only the inferior rectus motoneurons (IRMNs) supplying the medial compartment will have significant vertical sensitivity, whereas those supplying the lateral compartment will not. Fourth, during the same task, there was no change in contractility of the lateral superior oblique (SO) compartment, and therefore it is predicted that some superior oblique motoneurons (SOMNs) will not modulate during vertical vergence. We were able to test each of these predictions from analysis of motoneuron responses during these specific movements and found no evidence supporting central innervation leading to compartmental behavior. Some of these data were previously presented in abstract form (Adade, et al., ARVO 2022 e-abstract 2289).

Material and Methods

Subjects and Surgical Procedures

Data were collected from two young adult rhesus monkeys (*Macaca Mulatta*), aged seven and 10 years, with normal eye alignment. The animals underwent three surgical procedures to prepare them for behavioral and neurophysiological experiments. In the first surgery, a titanium head restraint post was implanted under aseptic conditions using isoflurane anesthesia (1.25%–2.5%).[18] In a second surgery, a recording chamber (21 mm diameter titanium cylinder) was implanted at a stereotaxic location that allowed full access to the abducens, oculomotor and trochlear nuclei. It was placed at a 20° angle to the sagittal plane to avoid large superficial blood vessels. In this same surgery, a scleral search coil was implanted underneath the conjunctiva of one eye.[19] The fellow eye was implanted with a scleral search coil during a third surgery. All surgical and experimental protocols were approved by the Institutional Animal Care and Use Committee at the University of Houston and were in strict compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research.

Behavior Task and Eye Movement Measurement

Before data collection, subjects were trained to fixate a high-contrast target rear projected onto a tangent screen 57 cm away and to perform various eye movement tasks specific to this study. The tasks included smooth-pursuit tracking of a horizontally and vertically moving sinusoidal target at 0.30Hz, ±15°, and asymmetric vertical vergence where one eye was stationary and only the other eye moved either upward or downward. Visual stimuli were generated using the BITS# visual stimulus generator (Cambridge Research Systems, Cambridge, UK) and Psychtoolbox 3[20] operated under computer control and presented using a DepthQ projector running at 120-Hz frame rate (Lightspeed Design, Inc., Bellevue, WA, USA). A detailed description of the dichoptic stimulus used to induce vertical vergence has previously been described.[21] Briefly, to create the vertical vergence stimulus, we used a full-field 50° × 50° stimulus pattern which comprised a dark background on which there was a bright central fixation cross (4° × 4°) and a sparse pattern of 50 dots (1° diameter each) placed randomly elsewhere. The stimulus pattern was presented dichoptically (using frame-sequential presentation to each eye in sync with liquid crystal display goggles) and vertical disparity was introduced by presenting one eye with the stationary pattern and the other eye with an identical pattern that was slowly displaced either upward or downward at the rate of 0.05 deg/s. To fuse the images being presented to each eye, the animal needed to generate an asymmetric vergence eye movement (i.e., one eye stationary and the other eye moving up or down). The large stimulus dot-pattern and the strategy of slow continuous introduction of vertical disparity helped to induce vertical vergence movements and furthermore to extend the range of vertical vergence such that modulations in motoneuron firing rate could be readily discerned. The horizontal and vertical positions of both eyes were recorded using the magnetic search coil technique (Primelec Industries, Regensdorf, Switzerland). We calibrated eye coil signals by monocularly presenting targets at several horizontal and vertical positions (±20°) and rewarding the animals with juice for maintaining fixation within a 2° window surrounding the target. Eye and target position data were processed with anti-aliasing filters at 400 Hz before sampling at 2.79 kHz with 12-bit precision (AlphaLab SNR system; Alpha-Omega Engineering, Nazareth, Israel). Eye position data were further calibrated offline and filtered using a finite

impulse response low-pass filter with a bandwidth of 0 to 80 Hz before further analysis.

Motoneuron Identification

All six extraocular motoneuron populations were identified by their stereotaxic locations in the midbrain, their increase in burst-tonic activity with eye movements in a preferred direction, and by electrical microstimulation.[22,23] Inferior rectus motoneurons (IRMNs) are the only population in the oculomotor nucleus with a downward on-direction. MRMNs in the right oculomotor nucleus are sensitive to leftward eye movements and had left burst-tonic activity (vice versa for MRMNs in the right OMN). Inferior oblique (IOMNs) and superior rectus motoneurons (SRMNs) in the OMN both have an upward on-direction but were distinguished by microstimulation. IOMNs innervate the ipsilateral eye, whereas the SRMNs innervate the contralateral eye. We applied electrical stimulation (400 Hz, 30–40 µA, 100 ms) in the area upon encountering an up-burst tonic motoneuron and observed which eye moved. Upward movement of the ipsilateral eye indicated the population as IOMNs, whereas upward movement of the contralateral eye indicated an SRMN population. We acknowledge that a few up-burst tonic cells in our population might be mis-assigned because anatomical studies have shown a very small percentage of IOMNs, and SRMNs projections are the opposite of what was previously stated,[23,24] but this did not have any implication for the interpretation of our data.

SOMNs increased activity with downward eye movement and are the only population found in the trochlear nucleus, which is caudal to the OMN. SOMNs also had an abducting component, which distinguished them from IRMNs in the OMN. The abducens nucleus, which contain LRMNs, was identified from its relative stereotaxic location caudal to the OMN and trochlear nuclei. Motoneuron single-unit data were recorded using epoxy-coated tungsten microelectrodes (1-5 Mohm Frederik Haer, Brunswick, ME, USA).

Experimental Design and Data Analysis

We recorded the firing activity of horizontal motoneurons during horizontal smooth-pursuit, vertical smooth-pursuit, and asymmetric vertical vergence. Cyclovertical motoneuron activity was recorded during only vertical smooth-pursuit and asymmetric vertical vergence. Multiple cycles of smooth-pursuit or trials of vertical vergence were averaged for analysis. This was especially useful for vertical vergence because of the small range of the eye movements.[25–29] Also for vertical vergence, only data acquired from three seconds before introducing vertical disparity until up to three seconds after the maximum disparity and where subjects were accurately performing the task were included in analysis. We excluded events where at least one eye was deviated from its intended target by more than 0.5°. Additionally, for the horizontal motoneurons, we excluded events where there was more than 1° of horizontal eye movement during the vertical vergence and vertical smooth pursuit tasks. Using the AlphaLab SNR system, we recorded motoneuron action potential data at a sampling rate of 40 KHz. Spike sorting was performed offline using template matching algorithm in Spike2 software. Time stamps corresponding to each motoneuron's action potentials were generated and used to compute its neuronal firing rate. To determine whether there are some MRMNs and LRMNs that contribute to vertical eye movements, we determined their horizontal and vertical position sensitivities using multiple linear regression analysis with the following first-order equation (Equation 1).[30–32] Data from horizontal and vertical smooth-pursuit were combined for the fitting procedure. We used Equation 2, which excluded horizontal position parameters, to fit the cyclovertical motoneuron data to determine whether there are some IRMNs, SOMNs, IOMNs, and SRMNs that do not contribute to generating vertical vergence.

$$FR(t-td) = Kv*VEP + Kh*HEP + Rv*VEV + Rh*HEV + C \quad (1)$$

$$FR(t-td) = Kv*VEP + Rv*VEV + C \quad (2)$$

In these models, *FR* is the instantaneous firing rate of the motoneuron, *VEP* and *VEV* are the innervated eye vertical position and velocity respectively. *HEP* and *HEV* are the innervated eye horizontal position and velocity, respectively. The regression coefficients *Kv, Kh, Rv* and *Rh* represent the motoneurons' vertical eye position sensitivity, horizontal eye position sensitivity, vertical eye velocity sensitivity and horizontal eye velocity sensitivity, respectively. The constant *C* is the motoneuron's firing rate at straight ahead gaze. Delays in neural processing were compensated for by the time shift expression (t − td), where td was fixed at 7 msec. MATLAB curve-fitting toolbox was used to perform the model fits. Motoneuron position and velocity sensitivities were considered statistically significant if the 95% confidence interval did not include zero and not significant if the confidence interval included zero.

Results

Horizontal Recti Motoneuron Activity During Vertical Smooth-Pursuit and Asymmetric Vertical Vergence

We recorded the neuronal activity of 26 MRMNs and 30 LRMNs from both sides of the brains in our two animals during the three different eye movement tasks. MRI studies of differential behavior of EOM compartments have suggested that specific MR and LR compartments contract significantly during certain vertical eye movements and therefore a significant sample of the MRMNs and LRMNs might show modulation of neural activity that is correlated with vertical eye movements. A sample MRMN recorded from the right OMN during these tested eye movement tasks is shown in Figure 1. As expected for a horizontal motoneuron, during horizontal smooth-pursuit, its firing rate increased on leftward eye movements and decreased on rightward movements with horizontal position sensitivity (*Kh*) of 5.26 spikes/s/deg (Fig. 1A). However, during vertical smooth-pursuit (Fig. 1B) and vertical vergence (Fig. 1C), there was no significant change in its firing rate with vertical position sensitivities (*Kv*) of 0.38 and 0.56 spikes/s/deg, respectively. These were in fact not significantly different from zero. Note that although the range of vertical vergence is small and there are frequent small conjugate changes in eye position during vertical vergence, the neuronal

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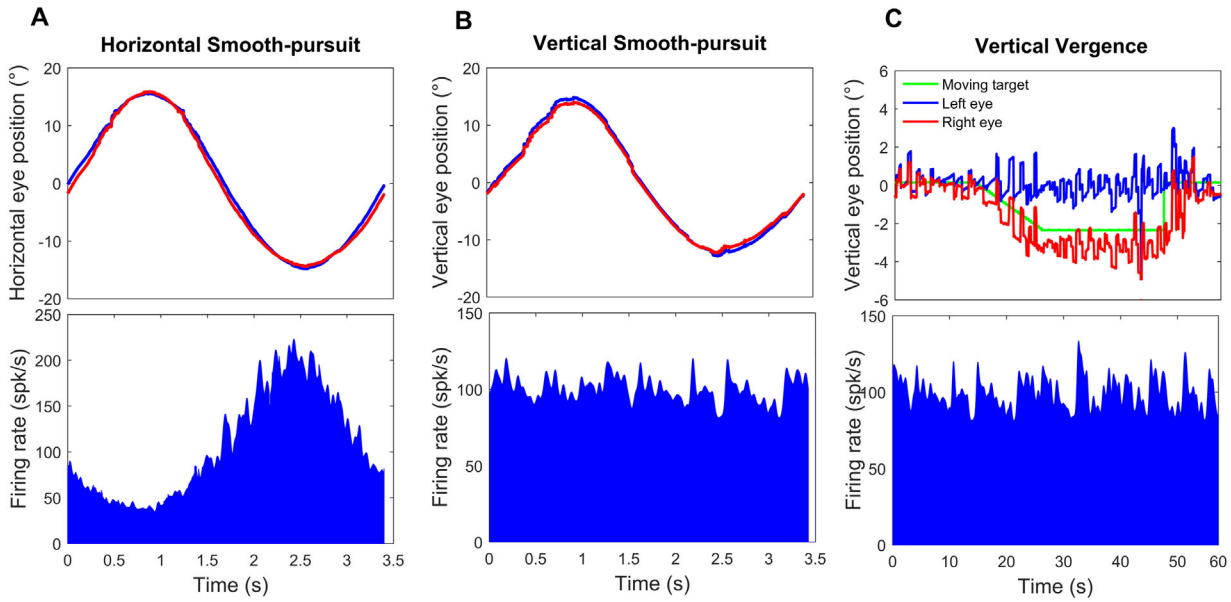

Figure 1. Discharge characteristic of an example MRMN recorded from the right OMN. Upward and rightward eye movements are indicated by upward (positive) deflections, whereas downward and leftward eye movements are indicated by downward (negative) deflections. *Red traces* indicate right eye position, *blue traces* indicate left eye position, and *green traces* indicate the moving stimulus location. During horizontal smooth-pursuit, the firing rate of the motoneuron increased on leftward eye movements, confirming it is a left burst-tonic cell, $Kh = 5.26$ spikes/s/deg (**A**). However, the firing activity of the cell did not modulate significantly during vertical smooth-pursuit with $Kv = 0.38$ spikes/s/deg (**B**). During asymmetric vertical vergence eye movements, where the innervated eye moved downward, there was no change in its firing activity, $Kv = 0.56$ spikes/s/deg (**C**).

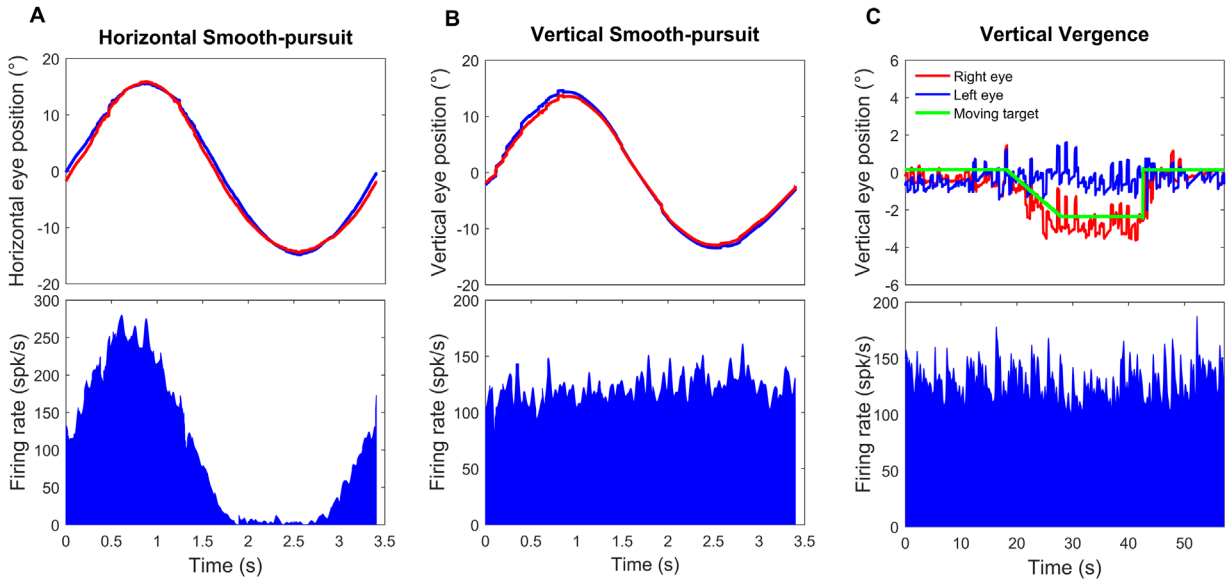

Figure 2. Discharge characteristic of an example LRMN recorded from the right abducens nucleus. For this abducens motoneuron, firing activity increased on rightward movement and decreased on leftward movement with $Kh = 8.24$ spikes/s/deg during horizontal smooth-pursuit (**A**). There was no significant change in the firing rate of the motoneuron during vertical smooth-pursuit, $Kv = 0.46$ spikes/s/deg (**B**), or vertical vergence, $Kv = 0.15$ spikes/s/deg (**C**).

modulation due to vertical vergence movement was clear for cyclovertical neurons (see Figs. 4B and 5B for examples) and clearly absent for horizontal recti motoneurons (Fig. 1C). The remaining 25 MRMNs behaved similarly, with no significant modulation during vertical smooth-pursuit or vertical vergence.

An example LRMN recorded from the right abducens nucleus is shown in Figure 2. The neuron modulated robustly during horizontal smooth-pursuit (Fig. 2A, $Kh = 8.24$ spikes/s/deg) but did not modulate significantly during vertical smooth-pursuit (Fig. 2B, $Kv = 0.46$ spikes/s/deg) or vertical vergence (Fig. 2C, $Kv = 0.15$ spikes/s/deg).

Figure 3A compares the position sensitivity during horizontal smooth-pursuit and vertical smooth-pursuit for all the horizontal motoneurons recorded. The vertical position sensitivities for all 26 MRMNs (red dots) were not statistically different from zero. Therefore none of the MRMNs recorded

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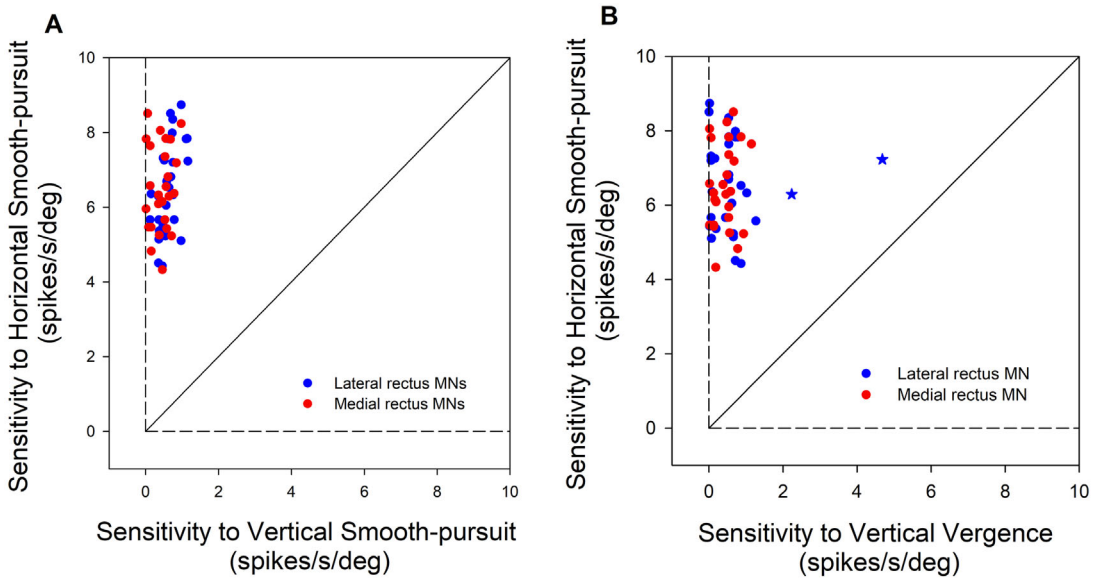

Figure 3. Comparison of position sensitivities during the different eye movement task. (**A**) Comparing position sensitivities of all horizontal motoneurons during horizontal smooth-pursuit and vertical smooth-pursuit. All 26 MRMNs and 30 LRMNs had no statistically significant vertical position sensitivity different from zero. (**B**) Comparison of position sensitivity during horizontal smooth-pursuit and vertical vergence. All 26 MRMNs did not have statistically significant vertical position sensitivity different from zero and only two of 30 LRMNs (*blue stars*) had a significant vertical position sensitivity.

provided any contribution to vertical smooth-pursuit. Also, vertical position sensitivity to vertical smooth-pursuit of all 30 LRMNs (blue dots) was not statistically different from zero. Once again, this indicates that that there is no contribution from the abducens motoneurons to vertical smooth-pursuit.

We also compared the position sensitivities during horizontal smooth-pursuit and vertical vergence for the horizontal motoneurons (Fig. 3B). Once again, all 26 MRMNs (red dots) showed no significant modulation during vertical vergence with zero vertical position sensitivity. The vertical position sensitivity during vertical vergence for 28 LRMNs (blue dots) were not different from zero. Only 2 LRMNs (blue stars) had a significant vertical position sensitivity during vertical vergence, but their vertical position sensitivity was much less than their horizontal position sensitivity (Kv = 2.2 vs Kh = 6.2 and Kv = 4.6 vs Kh = 7.2 spikes/s/deg). Due to the design of the vertical vergence task, the eyes moved very slowly and the regression coefficients relating firing rate and eye velocity were often insignificant. We therefore did not analyze and report velocity sensitivities.

Cyclovertical Motoneuron Activity during Asymmetric Vertical Vergence

IRMNs. We recorded 46 IRMNs from the left and right sides of the brain during vertical smooth-pursuit and asymmetric vertical vergence in order to test if we could identify a subpopulation of IRMNs that do not contribute to vertical vergence. An example of a left IRMN that modulated significantly during vertical vergence is shown in Figure 4. Its activity increased during asymmetric vertical vergence, which involved the innervated eye moving downward. The firing rate of 44 IRMNs significantly correlated with vertical eye position during vertical vergence with Kv values ranging from 1.2 to 10.8 spikes/s/deg (Fig. 4C). Only two IRMNs did not have a significant correlation between vertical eye position and their firing rates in this task. This shows that nearly all the IRMNs contributed to generating vertical vergence. Based on the size of the lateral compartment of the IR, which MRI studies suggested did not contract during vertical vergence,[14] we expected to find close to 50% of IRMNs population that would not modulate and have no significant vertical sensitivity during vertical vergence. However, only ~4% of our sampled neurons did not show significant position sensitivity.

SOMNs. We recorded 27 SOMNs from both sides of the brain during vertical smooth-pursuit and asymmetric vertical vergence. An example of a right SOMN that modulated significantly during vertical vergence is shown in Figure 5. Its firing rate increased during vertical vergence where the innervated eye moved downward. The firing rate of all 27 sampled SOMNs modulated during vertical vergence and Kv ranged from 3.1 to 15.8 spikes/s/deg (Fig. 4C). It is interesting to note that for most SOMNs, their Kv during vertical vergence was greater than that during vertical smooth-pursuit (6.3 and 4.8 spikes/s/°, respectively; $P < 0.001$). All the SOMNs have Kv showing significant vertical sensitivity; so we were unable to identify any SOMN that did not contribute to vertical vergence, in contradistinction to the predictions based on SO compartment behavior during vertical vergence.[14]

SRMNs and IOMNs. Although MRI studies did not show any observable differential compartmental behavior in the SR and IO during vertical vergence, we nevertheless recorded from 41 SRMNs and 35 IOMNs. Figure 6 compares Kv during vertical smooth-pursuit and vertical vergence for the recorded neurons. All the SRMNs and IOMNs had significant Kv during vertical vergence. We did not find any of these motoneurons that did not modulate significantly during vertical vergence.

Motoneuron recording locations. We attempted to sample motoneurons from different locations within

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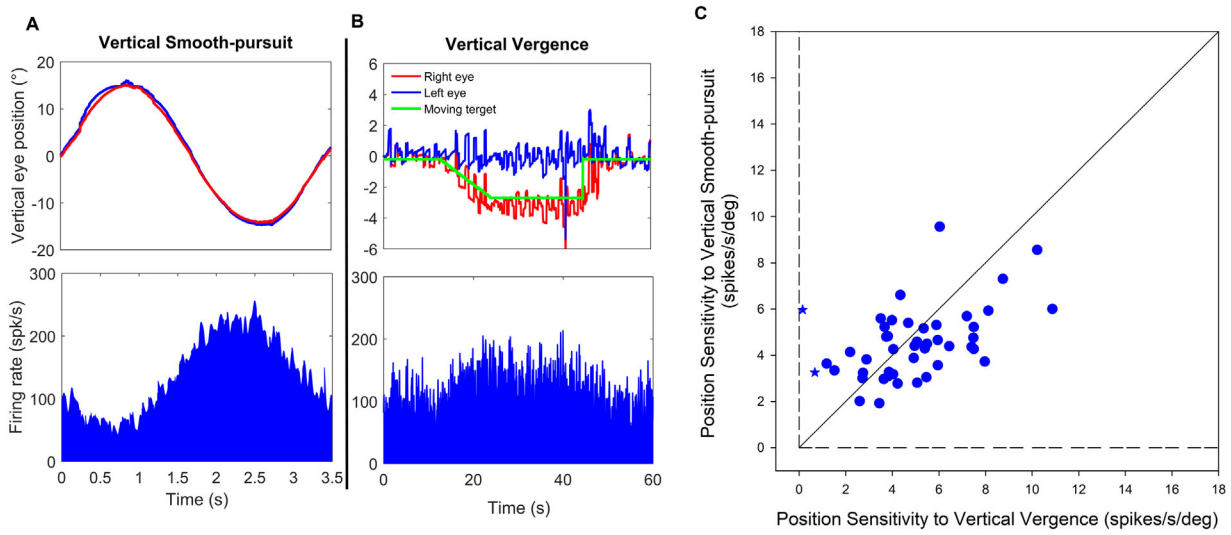

Figure 4. Discharge characteristics of IRMNs. (**A**) An example right IRMN whose firing activity increased on downward movement and decreased on upward movement during vertical smooth-pursuit with $Kv = 5.6$ spk/s/deg as expected for a down burst-tonic motoneuron. (**B**) During vertical vergence, its firing rate increased when the innervated right eye moved downward with $Kv = 6.3$ spk/s/deg. (**C**) The firing rate of 44 IRMNs significantly correlated with vertical eye position during vertical vergence. Only two IRMNs had Kv that was not significantly different from zero (*blue stars*).

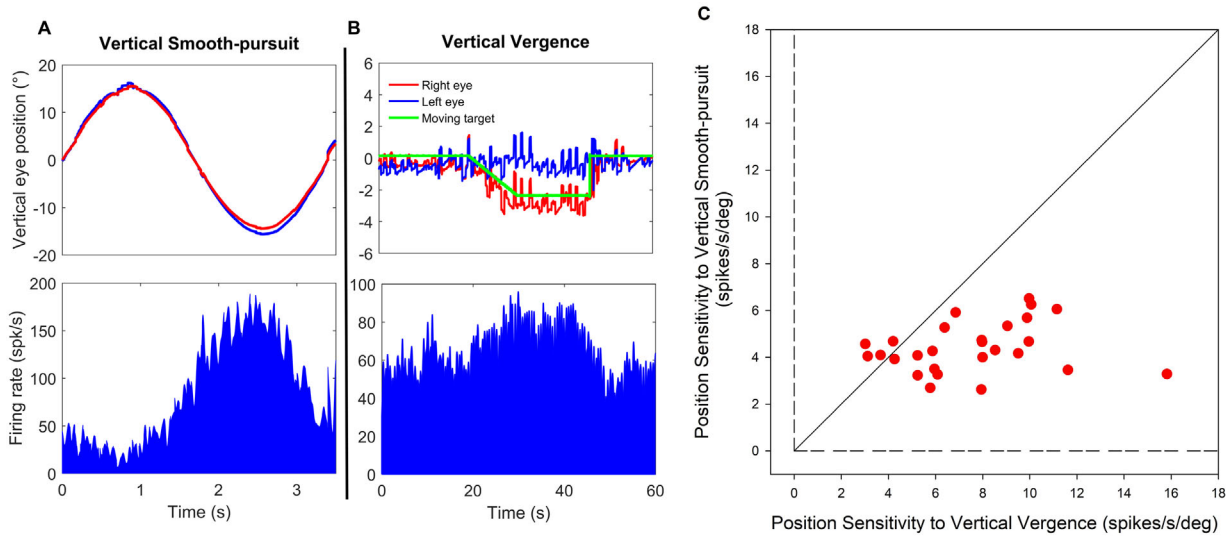

Figure 5. Discharge characteristics of SOMNs. (**A**) An example left SOMN whose firing activity increased on downward movement and decreased on upward movement during vertical smooth-pursuit, $Kv = 5.1$ spk/s/deg. (**B**) Its firing activity increased during vertical vergence when the innervated right eye moved downward, $Kv = 7.4$ spk/s/deg. (**C**) The firing rate of all 27 SOMNs significantly correlated with vertical eye position during vertical vergence. We did not find any SOMN whose Kv was not significant.

the abducens, oculomotor, and trochlear nuclei to avoid sampling error that could be relevant if motoneurons supplying the compartments were topographically segregated. In the absence of histological verification, Figure 7 shows the reconstruction of the recording sites of all motoneurons based on their recorded mediolateral and anterior-posterior locations at the surface of the recording chamber. Recording locations at the surface of the chamber are guided via a recording grid with 1mm spacing of grid holes. Because the anterior-posterior (AP) and mediolateral (ML) extent of the motor nuclei are relatively small (Abducens nucleus extent; ~1-2 mm AP, ~1-2 mm ML; OMN extent: ~4 mm AP, ~1 mm ML; Trochlear nucleus extent; ~1 mm AP, ~1 mm ML), many motoneurons were recorded at the same grid location over the repeated penetrations on different experimental days. However, plotting of recording location based on the surface grid hole location became complicated because it resulted in overlapping colored symbols. Therefore, to improve visualization of the colored symbols, a small offset is introduced at each grid location in Figure 7. Note also that driving the electrode a relatively long distance within the brain to record from neurons in the brainstem will lead to some additional variation of exact recording location within the area of interest and therefore works to our advantage of attempting to sample throughout the motor nuclei. As is evident from the figure, recording locations are distributed throughout the extent of the oculomotor, trochlear, and abducens nuclei on both sides of the brain.

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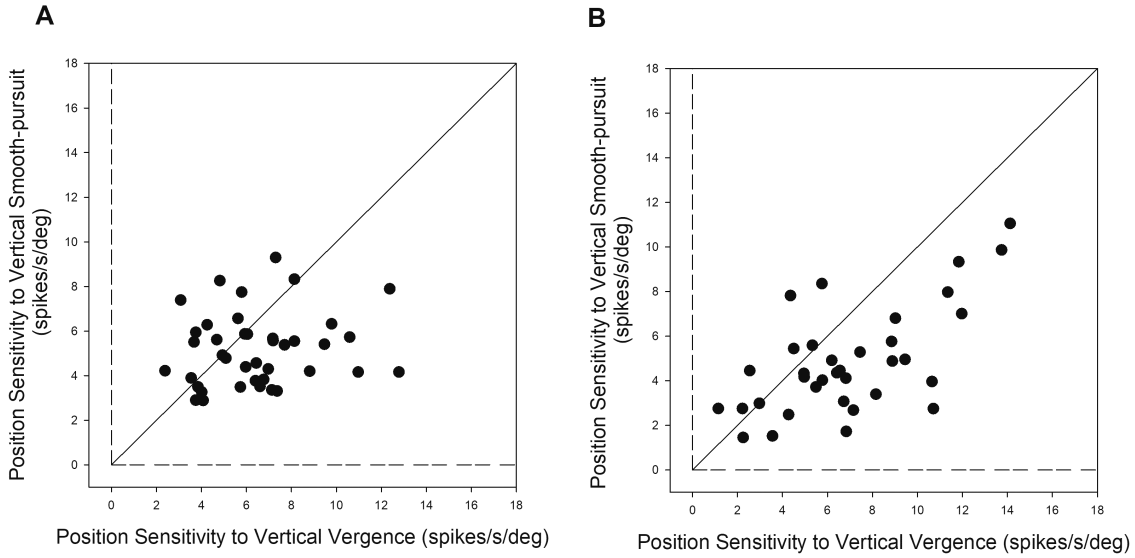

Figure 6. Comparing position sensitivity of SRMNs (**A**) and IOMNs (**B**) during vertical vergence and vertical smooth-pursuit. All 41 SRMNs and 35 IOMNs modulated significantly during vertical vergence with all having a significant *Kv*.

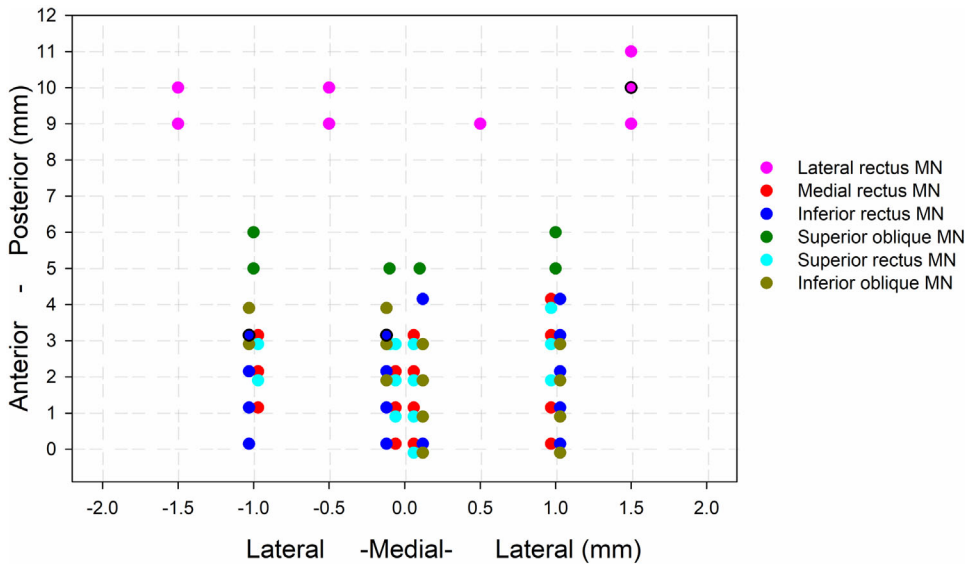

Figure 7. Reconstruction of motoneuron recording sites. *Top view* shows the mediolateral and anteroposterior recording sites of sampled motoneurons within the abducens, oculomotor and trochlear nuclei relative to each other. Negative numbers on the x-axis represent the left side of the brain and positive numbers are the right side of the brain. On the y-axis, the zero label arbitrarily represents the most anterior recording location of a motoneuron within the oculomotor nucleus. Positive numbers on the y-axis represent increasing posterior locations within the brain. Different colors represent different motoneuron subpopulations. For plotting oculomotor neuron recording locations, a small offset is applied to improve visualization of sub-types that were obtained via each grid hole. LRMN are offset by 0.5 mm to show their relative location away from the midline compared to oculomotor neurons. The recording sites of four motoneuron (two IRMN and two LRMN) that did not behave in the same way as the others during vertical vergence are represented with *black outline*. Note that in these same locations, we also recorded cells that behaved as the majority of the population.

Discussion

Da Silva Costa et al.[7] and Le et al.[9] suggested that most EOMs, with the exception of the SR, have two transverse compartments that can contract and relax independently during certain eye movements. The anatomical organization of the EOMs and the motor nerves supplying the muscles satisfy preconditions for the EOM compartments to be selectively innervated. They further hypothesized that the EOM compartments receive different control commands from separate motoneuron pools, to produce differential compartmental behavior in EOM described in their MRI studies.[7,9] The goal of the present study was to seek evidence for selective activity patterns for EOMs compartments by recording from extraocular motoneurons during some of the eye movements where the differential compartmental behavior was observed. However, the activity of extraocular motoneurons during vertical vergence and vertical smooth-pursuit do not support the theory that EOM compartments are selectively innervated. Consequently, we must conclude

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that other mechanisms downstream of the motoneurons might be responsible for the differential behavior of EOM compartments or that the observed differential compartmental behavior could be due to passive change in EOM cross-sectional area with gaze.

We recorded the activity of all six EOM motoneuron populations during vertical smooth-pursuit and vertical vergence, where MRI studies had revealed differential compartmental behavior in the MR, LR, IR, and SO. Although Demer and colleagues described a variety of differential compartmental behaviors during different eye movements, many of the differences are rather small and only evident as a small percentage change in a groupwise analysis of all subjects in a study. We specifically chose to test compartmental behavior by recording motoneurons during vertical smooth-pursuit and vertical vergence because these movements lead to either contraction in a horizontal muscle compartment or no contraction in a vertical muscle compartment and therefore led to readily testable hypotheses vis-à-vis motoneuron neural firing rate modulations. If the differential compartmental behavior is driven by oculomotor control, then we expected to find some MRMNs that would modulate significantly during vertical smooth-pursuit, some LRMNs that would modulate during vertical vergence, and some IRMNs and SOMNs that would not modulate significantly during vertical vergence. However, all 26 MRMNs did not modulate during vertical smooth-pursuit, and they had no significant vertical sensitivity. Only two of 30 (~7 %) LRMNs had a significant vertical sensitivity during vertical vergence. Because MRI studies indicated that there was significant contraction of the inferior LR compartment during vertical vergence, we expected close to 50% of the LRMN population would have significant vertical sensitivity. It is unlikely that the activity of such a small population could explain the observed contraction of the inferior LR compartment during vertical vergence. For the IRMNs, we expected about half of the neurons supposedly supplying the lateral IR compartment, which did not contract during vertical vergence, would show no modulation. However, we found only two neurons out of 46 IRMNs that did not modulate during vertical vergence. Again, this is less than the predicted 50% of the IRMN population. The finding of two LRMN with vertical sensitivity and two IRMN that did not modulate during vertical vergence is far too small a proportion to provide support to compartmental innervation but is puzzling all the same. The burst-tonic characteristics of these cells during conjugate movements were not different from the rest of the sample and the recording location was also among other neurons in the sample. Until better explanations can be hypothesized and tested, we can only attribute this very small minority to the vagaries of oculomotor biology. Last, we expected to find some SOMNs that would not modulate during vertical vergence, but did not find any, as all 27 SOMNs had a significant vertical position sensitivity that was even greater than the sensitivity during conjugate vertical smooth-pursuit. The greater SOMNs sensitivities during vertical vergence could be attributed to the generation of cyclotorsion that usually accompanies vertical vergence. SOMNs also show a decrease of activity during horizontal vergence because of cyclotorsion.[33] Moreover, our observation of different vertical sensitivities to vertical vergence vs vertical smooth-pursuit for cyclovertical motoneurons is analogous to findings for LRMN and MRMN sensitivities for horizontal smooth pursuit and horizontal vergence.[34–37] A summary of the differential compartmental behavior as reported by MRI studies, the predicted behavior of extraocular motoneurons and the observed motoneuron activity in this study are listed in the Table. These motoneuron behavior suggest that the EOM compartments are not selectively innervated i.e., they do not receive different motor command and thus, cannot explain the observed differential behavior of EOM compartments.[14,17] An argument against the negative findings in our study is that we may have simply missed or undersampled the motoneuron subpopulation with characteristics that would suggest the EOM compartments are selectively innervated. We could have missed the population supplying a specific compartment if there were some topographic organization within the motor nuclei and we had limited our recordings to a particular location. However, the motor nuclei were well mapped, and we recorded motoneurons from different locations within each motor nucleus, while also recording from both sides of the brain. In our opinion, it is unlikely that we would have missed or undersampled motoneurons that would suggest selective compartmental innervation across all the six extraocular motoneuron populations.

What downstream mechanisms could then explain the differential functional behavior of EOMs described by the studies of the Demer lab? EOMs are composed of different

Table. Summary of Predicted and Observed EOM Motoneuron Behavior During Eye Movements Where EOM Compartments Contracted/Relaxed Differently

Type of Eye Movement	Differential Compartmental Behavior Observed Via MRI	Predicted EOM MN Behavior	Observed EOM MN Behavior
Vertical duction (supraduction)	Significant contraction of superior compartment of MR	A subpopulation of MRMNs will have significant vertical sensitivity	None of the 26 MRMNs had significant vertical sensitivity
Vertical vergence (infraduction)	Significant contraction of inferior compartment of LR	A sub-population of LRMNs will have significant vertical sensitivity	Only 2/30 LRMNs had significant vertical sensitivity
Vertical vergence (infraduction)	Only the medial compartment of IR contracted whereas lateral compartment did not	A sub-population of IRMNs will have significant vertical sensitivity (~50%) whereas some will not (~50%)	Only 2/46 IRMNs did not have a significant vertical sensitivity
Vertical vergence (infraduction)	No change in contractility of lateral SO compartment	A population of SOMNs will modulate (~50%) whereas some will not modulate (~50%)	None of the 27 SOMNs had a significant lack of vertical sensitivity

fiber types that fall under two main groups: singly innervated twitch fibers (SIFs) and multiply innervated non-twitch fibers (MIFs). These are thought to mediate different eye movement based on their differential inputs[23,38–42] and the properties of the muscle fibers they supply.[2,43–45] However, recent studies suggest that all motoneurons fire for all types of eye movement, albeit at different rates for MIF and SIF motoneurons.[46,47] A systematic difference in the type and size of the muscle fibers in the EOM regions/compartments could explain the differential contractility. For example, a compartment with considerably more of the fiber type suited for a specific type of eye movement may exhibit more contraction than a compartment with less suited fibers, even if they receive the same innervation during that eye movement. Although we put this forward as a theoretical possibility, it is unlikely in our opinion, because studies that investigated EOM fiber types in different species have only reported a difference in fiber types between the orbital and global layers but did not report a difference along the EOM transverse section.[2,48–52] Moreover, it has been shown that the myofibers and fiber bundles in the EOM of humans and other species have significant connective tissue interconnections which allow force to be transmitted laterally.[53] This lateral dissipation of force supports the evidence for hysteresis where the sum of forces produced by single motoneurons are greater than the force measured when the whole nerve was stimulated.[36,54,55] Again, there are significant number of branched fibers and myomyous injunctions throughout the length and width of the EOMs.[45,52,56] Thus these features of EOMs suggest that the transverse regions may not be independent and that differential contraction of EOM compartments due to a central drive is unlikely.

It remains possible that the differential behavior of EOM compartments that was observed with MRI could be due to passive changes in muscle volume with gaze and not active differences in compartmental contraction. The EOMs are surrounded by orbital fat, which helps to stabilize the muscle path.[57,58] Studies have shown that the orbital fat can be deformed with changes in eye position despite its low elasticity and viscosity.[59] Such deformation of the orbital fat could lead to systematic passive changes in cross-section of the surrounded EOM, and perhaps misinterpreted as changes in muscle contractility. Moreover, Miller has suggested that passive changes in the shape of EOM cross-section caused by its pulley could explain differential compartmental behavior.[60,61] He suggested that changes in vertical gaze cause the horizontal EOMs to bend around their pulleys, which results in passive changes to the shape of the muscle's cross-section at the pulleys location. This could potentially explain why MRI studies showed certain compartments of LR and MR to be contracting during vertical eye movements and also account for the lack of a neuronal drive signal for observed compartmental changes.

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