Magnetic Resonance Imaging of Differential Compartmental Function of Horizontal Rectus Extraocular Muscles During Conjugate and Converged Ocular Adduction

Running Title: MRI of Extraocular Muscles in Convergence and Adduction

by

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Activity in horizontal rectus extraocular muscles (EOMs) was investigated by magnetic resonance imaging (MRI) of humans during asymmetrical convergence to a monocularly aligned target at 15 cm distance, or monocular fixation of afocal targets placed over a wide range of conjugate abduction through adduction. Cross sections and posterior partial volumes (PPV) of EOMs were determined from quasi-coronal image planes, and were separately analyzed in the inferior vs. superior compartments, defined by lines bisecting their maximum vertical dimensions. Both inferior and superior compartments of medial (MR) and lateral rectus (LR) exhibited contractile changes in PPV and maximum cross section for both asymmetric convergence and a comparable range of conjugate adduction. Both LR compartments, and the inferior MR compartment, exhibited similar decreases in contractility correlating with relaxation during both convergence and conjugate adduction. In contrast, the superior MR compartment exhibited roughly three times the contractility in conjugate adduction as in similar magnitude convergence. In the aligned eye that did not move during convergence, summed contractility in all compartments of MR and LR exhibited co-relaxation consistent with published EOM force measurements in this paradigm (Miller et al. 2002; Miller et al. 2011). The superior MR compartment also exhibited significantly greater contractility than the other compartments over the maximum achievable horizontal globe rotation from abduction to adduction. These findings suggest that the superior MR compartment is controlled differentially from the inferior compartment, and suggest that its activity is reduced during convergence as a component of generally altered extraocular mechanics.
Introduction

Ocular adduction implies rotation of the cornea towards the cranial midline; the opposite rotation is abduction, away from the midline. When adduction of one eye is accompanied by equal abduction of its fellow, the movements of both eyes are said to be conjugate versions. Convergence is disjunctive horizontal ocular rotation required to binocularly fixate proximally located targets. It is physiologically possible for the same adducted position of an eye to be the result of a conjugate pair of ocular rotations, or the result of pure convergence of that eye without movement of its fellow, or some combination of version and vergence eye movements.

Presumably in each case, both eyes would rotate until the sum of adducting and abducting forces, including passive connective tissue forces, reaches zero. Active contributions to such forces would conventionally be expected to be closely related to the firing behavior of the motor neurons innervating the extraocular muscles (EOMs), particularly the medial (MR) and lateral rectus (LR) EOMs that generate the overwhelming majority of active horizontal oculorotary force.

Although the foregoing suggests that motor neurons innervating MR and LR would exhibit similar, reciprocal discharges during convergence and conjugate adduction, experiments in monkeys have consistently shown otherwise. Rather than demonstrating the expected decrease in firing in convergent adduction 100% of that in conjugate adduction, the relative abducens neuron firing rate decrease during converged adduction has been observed in variously to be 62% (Mays and Porter 1984), 50% (Gamlin et al. 1989), and 50% (Miller et al. 2011). This is also the case for abducens interneurons (Gamlin et al. 1989). Moreover, in monkey abducens nerve, modulation of most units correlates with monocular movement of either eye (Zhou and King 1998). Even with the ipsilateral eye immobile, many LR motor neurons paradoxically
increase firing during contralateral eye convergence (Zhou and King 1998). These findings predict MR and LR co-contraction in convergence that was not observed by Miller et al., who obtained measurements from MR and LR force transducers in monkeys during asymmetrical convergence (Miller et al. 2002; Miller et al. 2011). Asymmetrical convergence aligns one eye on the same target for both near and distant viewing, avoiding confounding eye position changes (Allen and Carter 1967; Rashbass and Westheimer 1961; Steffen et al. 2000). Rather than MR and LR co-contraction in asymmetrical convergence, Miller et al. observed the opposite: slight force reduction in both EOMs (Miller et al. 2002). Miller, Davison, and Gamlin then recorded MR and LR motor neurons simultaneous with both EOM forces during asymmetrical convergence, confirming in abducens neurons that the ratio of vergence to conjugate sensitivity is one half (Miller et al. 2011). Yet, the same ratio was 1.53 in MR motor neurons, implying stronger MR firing in asymmetrical convergence than in conjugate adduction (Miller et al. 2011). Notwithstanding this, simultaneous MR and LR force measurements again confirmed lower forces in both EOMs during asymmetrical convergence (Miller et al. 2011).

Thus, study of EOM forces and motor neurons during convergence has produced definitively contradictory findings under the assumption that there is a simple relationship between force in horizontal rectus EOMs, and the firing rate of their motor neurons. A possible resolution for this paradox might be an intermediate phenomenon altering the assumed direct relationship between motor neuron firing and development of oculorotary force. Orbital magnetic resonance imaging (MRI) has become useful to investigate aspects of EOM action. This noninvasive technique is suitable for characterizing the function of human EOMs during visual fixation. Unlike studies of force, MRI indicates contractility directly by morphologic changes associated with EOM contraction. Miller introduced MRI for quantitation of rectus
EOM contractility by analyzing changes in cross sectional area distribution associated with EOM shortening (Miller 1989). When applied to pathology, Miller's approach demonstrates atrophy and reduced contractility in denervated human rectus EOMs (Demer 2003; Demer and Miller 1999), as well as in normal and palsied superior oblique (SO) (Clark and Demer 2011; Clark et al. 1998; Demer and Miller 1995; Jiang and Demer 2008; Kono and Demer 2003; Kono et al. 2009) and inferior oblique (IO) EOMs (Demer et al. 2003b; Ela-Dalman et al. 2008; Kono and Demer 2003). Validity of MRI for EOM evaluation is supported by the observation that after trochlear neurectomy in monkey, both post-mortem MRI and histology show similar SO atrophy to that demonstrated by MRI in living humans (Demer et al. 2010). Moreover, the aligned convergence task can be performed by humans during MRI under viewing conditions similar to those of Miller et al.'s monkey experiments (Miller et al. 2002; Miller et al. 2011). Such human experiments by Demer et al. demonstrated extorsion of the rectus pulley array during aligned convergence, and absence of retraction of the aligned eye inconsistent with increased summed MR and LR forces (Demer et al. 2003a). This MRI finding, while supportive Miller et al.'s EOM force data (Miller et al. 2002; Miller et al. 2011), is nevertheless inconsistent with the foregoing body of motor neuron data.

Compartmentalization has recently emerged as an additional feature of EOM function that might be relevant to convergence. Because human rectus EOM tendons are about 10 mm wide (Apt 1980; Apt and Call 1980; Demer 2009), the center of force of such idealized actuators is generally not at the anatomical midpoint of the tendon's insertion on the globe, but rather shifts with the transverse force gradient in the tendon that is a function of factors including differential stretching arising from bending (Miller and Robinson 1984; Robinson 1975). Rectus EOMs also include what would at first seem a surfeit of fibers (Goldberg et al. 1998; Goldberg et al. 1997).
If rectus EOM force were concentrated at different points along the transverse dimension of its tendon, mechanical effects would vary correspondingly. Potential for physiological alteration the locus of force application was revealed by three dimensional histological reconstructions in humans and monkeys showing a non-overlapping pattern of terminal intramuscular LR innervation (Peng et al. 2010) and MR (da Silva Costa et al. 2011; Peng et al. 2010). Prior to entry into each horizontal EOM, its motor nerve divides into superior and inferior branches supplying non-overlapping distributions forming segregated neuromuscular compartments maintained along the EOM’s entire length (da Silva Costa et al. 2011; Peng et al. 2010). These neuromuscular compartments contain roughly parallel EOM fibers having only sparse lateral interconnections (Demer et al. 2010; Lim et al. 2007). At the insertional tendon, local rectus EOM regions also exhibit considerable mechanical independence. As little as 5% of passive tensile loading upon half of an isolated bovine rectus EOM tendon is typically reflected in the loading of the opposite half tendon, suggesting appreciable mechanical independence between adjacent EOM fibers (Shin et al. 2012). If anatomically distinct neuromuscular compartments of horizontal rectus EOMs were to contract independently, force changes in the compartments might offset one another. An increase in inferior compartment tension hypothetically might be offset by a greater decrease in superior compartment tension to produce net relaxation; the apparent relationship of motor neuron firing to net tension would depend upon the relative sampling of motor neurons in the pools innervating the two compartments.

Although it has been common to study EOM function by measuring developed tension when maintained at a fixed length (isometric) (Lennerstrand et al. 2006), muscle contraction has traditionally also been studied by measuring changes in length under fixed load (isotonic) (Wilkie 1956). Moreover, EOM behavior can also be studied during release from a set initial
load (McLoon et al. 1985). Multiple measures of muscle behavior are considered to reflect "contractility" (Clausen 2003). A systematic study of whole human horizontal rectus EOM contractility has demonstrated high correlations to conjugate horizontal duction angle for both maximum cross sectional area and posterior partial volume (PPV), a morphometric that integrates area over multiple contiguous cross sections and thus can be considered a non-invasive indicator of contractility (Clark and Demer 2012c). It is now feasible to employ MRI to study differential compartmental contractility in humans. Differential contraction of the LR inferior compartment (LRi) has been demonstrated by MRI during ocular counter-rolling induced by head tilt (Clark and Demer 2012a), and in the superior MR compartment (MRs) during vertical duction (Demer and Clark 2013).

The present study employed MRI to seek evidence of possible differential compartmental contractility in the superior vs. inferior compartments of the horizontal rectus EOMs during asymmetric convergence similar to the paradigm employed by Miller et al. (Miller et al. 2002) (Miller et al. 2011). For comparison, conjugate horizontal duction was also examined.
Glossary

AIN – abducens interneuron
CN3 – oculomotor nerve
CN4 – trochlear nerve
CN6 – abducens nerve
EOM – extraocular muscle
FOV – field of view
GL – global layer
LRi – inferior compartment of lateral rectus muscle
LRs – superior compartment of lateral rectus muscle
MRi – inferior compartment of medial rectus muscle
MRI – magnetic resonance imaging
MRs – superior compartment of medial rectus muscle
OL – orbital layer
SM – smooth muscle


Materials and Methods

Subjects. Healthy adult subjects were recruited by advertisement and gave written informed consent according to a protocol conforming to the Declaration of Helsinki and approved by the Institutional Review Board (IRB) for Protection of Human Subjects at the University of California Los Angeles. Volunteers for functional testing underwent eye examinations verifying normal corrected visual acuity, normal ocular versions, orthotropia at near and distance, and 40 arcsec contour stereopsis by crossed polarization testing at near (Titmus). Volunteers were compensated financially for participation. In this paper, an "MRI scan" is considered to be the acquisition of a set of contiguous image planes during a time interval of up to 3.7 min while the subject was instructed to continuously perform a designated visual fixation task. Routinely, 10 - 16 such MRI scans can be performed during a single session lasting up to 90 min, although some scans must necessarily be performed at low resolution for anatomical localization to enable higher resolution views, and scans were repeated as necessary in the event of technical problems.

Subject numbers, ages, and numbers of MRI scans are listed for each study in the Table. Subjects participating in the "asymmetric convergence" study were selected for ability to maintain convergence to a target 15 cm away. The asymmetric convergence experiment was difficult for subjects to perform, requiring at least 7 MRI scans for one eye converging, and at least five more for the fellow eye converging. Scans were immediately repeated if images showed that target positioning or convergence effort was not appropriate. Twenty subjects initially appeared qualified to perform the asymmetric convergence experiment, but only 11 who were aged 23±4 yrs (mean ± standard deviation, SD) could converge under actual MRI conditions to provide interpretable data from the approximately 220 scans performed. These
were matched to 11 additional subjects aged 24±5 yrs who contributed to the "matched adduction" data set. These subjects were imaged in central gaze and in conjugate adduction to angles individually matched post hoc to the adduction achieved during convergence by each participant in the convergence experiment, requiring the analysis of an additional 33 scans. A further group of 15 subjects contributed to the "full horizontal duction" data set. These subjects, aged 23±5 yrs, underwent a total of 77 MRI scans covering a range of conjugate horizontal gaze ranging from maximal abduction to maximal adduction, including 3–5 fixation positions per subject.

Finally, a group of 75 subjects contributed a total of 150 quasi-coronal MRI image sets in unconverged, central gaze for quantitative analysis of the distribution of the inferior and superior compartments of the LR, based upon the visualization of a demarcating anatomic feature visible on MRI. Of these, anatomical features demarcating the putative border between the inferior and superior LR compartments were distinguishable in 30 orbits of 23 subjects aged 31±16 yrs that were then analyzed to determine the proportions of LR cross section in the two compartments, respectively.

**Visual Stimuli.** As detailed elsewhere (Demer et al. 2003a), subjects wore a facemask containing an embedded surface coil array (Medical Advances, Milwaukee, WI) as they lay supine looking upward. For MRI involving convergence, a plastic, semi-transparent mirror was mounted with its center 15 cm above the eyes and angled 45° to the vertical, reflecting the image of a distant, flashing red light emitting diode (LED) across the room 600 – 800 cm away (depending on the individual MRI installation) to appear virtually above the subject in centered vertical position. The end of a fine optical fiber was centered over the half mirror, so that when an LED remotely illuminated the fiber end, a point target was visible 15 cm away. Near and far
targets were then aligned to one eye by moving the far target laterally, but only illuminated
alternately depending upon the testing condition. With the far target illuminated, both eyes were
in conjugate gaze ~ 11° opposite the side of the aligned eye. With the near target illuminated, the
converging eye adducted without changing vertical position. Depending upon target placement,
either eye could fulfill the role of aligned or converging eye, and in each imaging session that
lasted up to 90 min, an attempt was made to have both eyes do so sequentially. In initial images,
the right eye was assigned to be aligned, while the left eye converged. Later in the same session,
the distant target was moved so that the left eye was aligned while the right eye converged.
Successful performance of the convergence task was ascertained from actual MRI images. As it
happened, no subject could successfully perform the task with both eyes in the aligned role, but
each subject included in analysis did so for one of their two eyes.

Prior to MRI, each subject's ability to sustain convergence was verified by visual
inspection of eye position through the half mirror. Subjects were included for data analysis only
when axial MRI imaging demonstrated robust adduction of the converging eye; 9 additional
normal subjects, not contributing to data reported here, failed to achieve adequate convergence
as indicated by parallel lines of sight on axial MRI, and/or failure of adducting shift of the globe-
optic nerve junction as indicated on quasi-coronal MRI. Data were analyzed for converged scans
only when positions of the globe-optic nerve junction confirmed convergence. Nevertheless, the
actual convergence angle achieved varied among individuals and trials, and was measured from
the images themselves for each trial that was analyzed.

For study of conjugate duction, subjects monocularly viewed a single, fine illuminated
optical fiber about 2 cm distant, placed at chosen horizontal eccentricity but always in centered
vertical position. While this afocal, monocular target was physically "near," it had no optical
features of proximity; control experiments have verified that this afocal target does not elicit
convergence.

**Magnetic Resonance Imaging (MRI).** Under prospective IRB approval, MRI sets of
ophthalmic examination-verified, normal living human orbits have been collected per protocol
for research purposes under carefully a variety of documented task conditions since March 1995.
High-resolution, T1 (Demer 2003) or T2 fast spin echo (T2FSE) (Demer and Dusyanth 2011)
weighted MRI was performed using a 1.5 T General Electric Signa (Milwaukee, WI) scanner
using a surface coil array (Medical Advances, Milwaukee, WI) and techniques elsewhere
described (Clark and Demer 2012a; Demer et al. 2003a). We employed technique for minimizing
high resolution scan duration as published by our group (Demer and Dusyanth 2011). Subjects
wore earplugs during imaging. Between scans, rest and instruction periods of 1-3 minutes were
provided during which subject comfort was verbally ascertained. Initially, a low resolution
triplanar scan was used to localize sets of contiguous, 2 mm thick axial image planes employing
a 256x256 matrix over a 9–10 cm field of view (FOV), minimally sufficient to include both
orbits at 350–390 microns pixel resolution. Axial images were repeated for near and far targets,
confirming in successful cases that appropriate convergence changes were attained by each
subject (Fig. 1). When the T1 sequence was employed as in Fig. 1, the paramagnetic MRI
contrast agent gadodiamide (0.1 mmol/kg total dose) was given intravenously before some
subsequent scans (Oh et al. 2001). Morphological information is similar in T1 and non-contrast
T2FSE scans (Demer and Dusyanth 2011). For both far and converged target viewing, sets of
17-19 contiguous quasicoronal images were obtained in planes 2 mm thick and perpendicular to
the axis of each orbit separately using a 256x256 matrix over an 8 cm square FOV, giving 313
Digital MRI images were quantified using the program *ImageJ64* (W. Rasband, National Institutes of Health, Bethesda, MD, http://rsb.info.nih.gov/ij/, 1997-2009). For analysis of LR compartmental distribution based upon anatomical features, quasi-coronal images were examined throughout both orbits to identify fissures or bright structures demarcating the LR inferior (LRi) from superior (LRs) compartments in any image plane (Fig. 2.). On the images, lines were then drawn by inspection paralleling the maximum transverse (usually vertical) extent of that LR cross section, and the proportion of each line inferior to the anatomic demarcation was determined. Because of the generally parallel, longitudinal arrangement of EOM fibers, this proportion was taken to reflect the entire EOM.

For functional studies of convergence and adduction, digital MRI images were quantified using *ImageJ64* and customized image analysis programs in MatLab® (MathWorks, Boston, MA, 2011). Eye position was determined by location of the optic nerve centroid relative to the orbital centroid at the globe-optic nerve junction (Clark et al. 1997). Each rectus EOM was manually outlined and cropped to include only its belly (Clark and Demer 2012b; c), avoiding inclusion of adjacent non-muscular structures. The remaining steps in analysis were automated. The angle of a linear best-fit line through the largest transverse dimension of the cropped EOM was computed (Clark and Demer 2012a) and the entire image was rotated to align that best-fit to vertical. Superior and inferior EOM compartmental areas were calculated by separately determining cross sectional area above and below the perpendicular bisector to this best-fit line (Clark and Demer 2012a) Cross sections in the four contiguous image planes -4, -5, -6, and -7 (8...
to 14 mm posterior to the globe-optic nerve junction) were summed and multiplied by the 2 mm slice thickness to form PPVs for the respective compartments.

Contractility of EOMs was inferred from longitudinal cross sectional area distributions. For this purpose, image plane sets were aligned on the globe-optic nerve junction for far target viewing, permitting averaging over multiple subjects for each EOM for comparable viewing conditions. Location of the cross section peak reflects contractility but may not be an optimal quantitative measure of horizontal rectus EOM contractility (Clark and Demer 2012c). In any material, passively applied force (stress) is directly related to deformational shape change (strain) from the initial shape. Because mechanical strain should similarly reflect EOM force and is defined in proportion to undeformed dimensions, contractility was defined as the percentage change in PPV from that measured in the reference eye position. The reference position was taken as distant gaze for convergence experiments, and central gaze for non-converged gaze experiments.

Change in PPV has been demonstrated to be a robust correlate of duction angle for horizontal rectus EOMs, accounting for more than 85% of variance in duction of multiple individual subjects, and at least 97% of variance within individual subjects (Clark and Demer 2012c). Similar correlations for maximum cross sectional area are robust but not as high as for PPV (Clark and Demer 2012c). While maximum cross sectional area was also analyzed and supported similar conclusions to those based upon PPV, maximum cross section data are not reported in detail here.

While every rectus EOM contains an oculorotary GL and an OL that translates its pulley, the borders between these layers cannot be discerned for horizontal rectus EOMs. The automated parsing of LR and MR into inferior and superior compartments therefore included both GL and
OL contributions. Linear regressions, analyses of variance (ANOVA), and Gaussian fits to data were performed using GraphPad Prism (GraphPad Software, La Jolla, CA, USA).

Results

Compartmental Distribution in the Lateral Rectus. Of 75 normal subjects imaged, 30 orbits of 23 subjects exhibited fissures or horizontal lines demarcating putative borders between LRi and LRs (Fig. 2). When such demarcations were visible in multiple image planes, as in Fig. 2 -18 to -14 mm, the relative proportions of LRi and LRs appeared similar in all planes. Figure 3 illustrates the frequency with which the relative proportion in LRi fell within bins of 0.05. The distribution was slightly leftward skewed, but the mean was 0.49—0.10 (SD), giving a 95% confidence interval of 0.3–0.7. The confidence interval about roughly 50% appears reasonable, since the proportions in only two of 30 orbits were outside that interval.

Since no anatomic demarcation in the LR was visible by MRI in the majority of orbits analyzed for functional experiments, and since corresponding demarcation was almost never identifiable for the MR, anatomic features were not used to identify compartmental borders. For both LR and MR, the compartment border was assumed, based upon histological reconstructions (da Silva Costa et al. 2011), to be half the greatest vertical dimension of each EOM. If this assumption were incorrect for any individual subject, functional changes in one compartment would be misattributed to the other, reducing apparent differential function. The overall effect of such errors would be to reduce the ability of the experiment to detect differential compartmental contraction, but would never falsely suggest existence of differential contractility.

A more conservative approach to data analysis was also performed that excluded the entire 90 or 95% confidence interval for the anatomical compartmental demarcation as
determined in Fig. 3. While these analyses supported the conclusions to the 50% division for which data are reported in detail here, exclusion from analysis of the central 60% of each EOM cross section resulted in higher variance. This effect is presumably due to the small EOM cross section remaining for analysis relative to fixed image pixel size.

Contractility was evaluated morphologically, by the distribution of cross sectional area along the length of the horizontal rectus EOMs. Figure 4 plots mean (± SEM) LR and MR cross section for the adducting eye or the eye converging to near while the fellow eye remained aligned, as a function of anteroposterior position in the orbit from 2 mm thick image planes, referencing the image plane containing the junction of the globe and optic nerve as plane zero. Although target position was uniform, the actual angle of asymmetric convergence that was achieved varied somewhat and is considered separately in a later section. Rectus EOMs have small cross sections near their origins in the deep orbit, reach maxima in mid-orbit, and become attenuated as they transition into non-contractile tendons near the scleral insertions. Quantitatively, this is seen in Fig. 4 as a peak in the cross sectional area distribution for distance viewing at planes -2 to -4, corresponding to 4 to 8 mm posterior to the globe-optic nerve junction. For the MR in central gaze (Fig. 4A) or far gaze with about 11° adduction (the control condition for the convergence experiment, Fig. 4C), the inferior compartment MRi was larger than superior compartment MRs. In both conjugate adduction (Fig. 4A) and in the eye converging to near while the fellow eye remained aligned (Fig. 4C), the peak cross section increased and shifted posteriorly. For the LR in central (Fig. 4B) or far gaze with about 11° adduction (Fig. 4D), the inferior compartment LRi had larger cross section than the superior compartment LRs. Opposite to the morphological change of the MR, in both conjugate adduction
and in the eye converging to near while the fellow eye remained aligned, peak LR cross section decreased and shifted anteriorly.

Gaussian curves gave reasonable fits to the EOM cross section distributions for all experimental conditions (Fig. 4), with coefficients of determination of 0.6 or more for the MR and LR in adduction, but 0.3 – 0.5 for the LR in conjugate adduction, and for both EOMs in asymmetric convergence with the fellow eye aligned. Inspection of the data suggested that the peaks of the Gaussian curves closely matched the data peaks. This impression was tested by the statistics of the Gaussian fits. By the replicates test (Bates 1988), all 16 Gaussian curves represented in Fig. 4 can be considered adequate representations of the data (P > 0.05). For both compartments of the MR and the LR, and for both adduction and for convergence, the null hypothesis that one identical Gaussian curve can represent all four data sets (MRi and MRs, or LRi and LRs, for two eye positions each) in each panel of Fig. 4 A - D is rejected at P < 0.0001. Similarly, for the data of each panel of Fig. 4A - D, the null hypotheses are rejected that all four of the foregoing data sets can be represented by the same Gaussian peak cross section, or at the same image plane location (P < 0.0001 for both). However, in no case did the breadth (SD) of the Gaussian fit differ significantly by muscle (MR or LR), compartment (inferior or superior), or eye position (P > 0.05). To summarize these statistical analyses, Gaussian curves are good representations of the cross sectional area distributions of both compartments of both horizontal rectus EOMs, and these distributions change independently for the compartments for both convergence and adduction in the paradigms studied here.

The position of the peak of each Gaussian cross sectional area curve, plotted as a function of 2 mm thick image plane number ±SEM, is illustrated in Fig. 5. It may be seen that for both conjugate adduction and asymmetric convergence with the fellow eye aligned, the cross section
distribution in each horizontal rectus compartment peak shifted posteriorly by about 1 image plane (2 mm) during contraction and anteriorly by about 1 image plane during relaxation.

The percentage change in PPV is plotted in Fig. 6 for the MR and LR for similar angles up to 25° conjugate adduction (Fig. 6A) and for the eye in asymmetric convergence with the fellow eye aligned (Fig. 6B). In each case, an adequate range of duction angles permitted linear regression against PPV. Linear regression was performed to compute means and standard errors (SEs) for regression slopes. It is evident from the slopes of the regression lines for both conjugate adduction (Fig. 6A) and asymmetric convergence with the fellow eye aligned (Fig. 6B) that contractility was similar for both LRi and LRs, and that these slopes ranging from 0.69–1.40%/deg were similar for both conjugate adduction and asymmetric convergence with the fellow eye aligned. Coefficients of determination $R^2$ for the two LR compartments ranged from 0.24 for LRs in asymmetric convergence, to 0.54 for LRi in conjugate adduction. The extra sum of squares F test did not reject the hypothesis that the linear regression, including slope and intercept, was the same for LRi and LRs in both conjugate adduction and asymmetric convergence ($P=0.5526$).

Behavior of the MR differed from that of the LR. The extra sum of squares F test rejected the null hypothesis of the same regression equation (both slope and intercept compared, $P=0.0004$) or slope alone ($P=0.012$) for MRi and MRs for both conjugate adduction and asymmetric convergence, in favor of the alternative hypothesis that the regressions differ for each compartment and condition. Regression slope is considered the measure of contractility. During conjugate adduction, the slope for MRs, $2.99\pm0.52%/deg$, was not significantly higher than the slope for MRi, $1.88\pm0.54%/deg$ ($P>0.1$, Fig. 6A). However, during conjugate adduction, contractility in MRs was significantly about three times that during asymmetric...
convergence with the fellow eye aligned at 1.06±0.31 %/deg (P==0.0018), although not
significantly different from the value of 0.65±0.21 %/deg in MRi(P>0.25). Contractility in MRi
was not significantly different in conjugate adduction vs. asymmetric convergence (Fig. 6,
P>0.1). In conjugate adduction, regression demonstrated that contractility in MRs at 2.99±0.52
was significantly greater than in LRs at 1.40±0.43 (P=0.0252); corresponding contractilities for
MRi and LRi did not differ significantly (P>0.3).

In view of the differential compartmental contractility in the MR demonstrated for the
relatively narrow range of adduction angles achieved during asymmetric convergence with the
fellow eye aligned, compartmental analysis of horizontal rectus contractility was also performed
in a larger group of 15 subjects who in multiple MRI scans fixated target positions ranging over
approximately ±30° and thus included large angles of abduction in addition to adduction. Linear
regressions of PPV against duction angle are illustrated in Fig. 7, with agonist angles for both
MR and LR represented as positive for comparison. The extra sum-of-squares F-test rejected the
null hypothesis that a single regression slope, or even a single regression equation, could
describe both the inferior and superior compartments of both MR and LR (P<0.0001). For each
of the four individual compartment regressions, coefficients of determination R² exceeded 0.83.
In this large data set, contractility of LRs at 1.52±0.08%/deg was not significantly different from
the 1.38±0.06%/deg value for LRi (P>0.1), but contractility of MRs at 1.84±0.08%/deg was not
only markedly greater than the 1.28±0.06 value for MRi (P<0.0001), but also greater than for
LRs (P<0.0038). Thus, evaluation over a broad range of horizontal duction confirmed that MRs
has highly significant 32% greater contractility than the pooled values for MRi, LRi, and LRs.

Since by design the aligned eye moved very little during asymmetric convergence by its
fellow, it was not possible to perform regression of PPV against duction angle. However, EOM
cross sections for the aligned eye are plotted in Fig. 6B (near the origin) for both compartments of the MR and LR. For MRi and MRs, the small mean change in PPV during convergence did not significantly differ from zero. However, for both LRi and LRs, larger negative contractility values of -5.65±5.04 and -5.30±6.21%/deg were significantly sub-zero (P<0.01). Variance in the foregoing computations includes intersubject variations, the effects of which, analogous to a paired t-test, can be minimized by calculating the paired sums for the inferior and superior compartments within individuals. The values for MRi plus MRs and LRi plus LRs, summed in individual orbits, had significantly negative average values of -7.00±6.54 and -5.75±3.01%/deg (P<0.01). These negative values demonstrate relaxation of both compartments of LR and MR during convergence, and therefore exclude co-contraction of MR and LR during convergence.
Discussion

Asymmetric convergence has been useful in human behavioral studies as well as animal studies of neural activity, and now has proven valuable for MRI investigation of horizontal rectus EOM compartmental actions during sustained human convergence and sustained, unconverged duction. In both paradigms involving sustained fixation, maximum cross sections of the inferior and superior compartments of contracting EOMs increased and the points of maximum cross section shifted posteriorly. Quantitative effects, however, differed for MRs compared in the other three horizontal rectus compartments. During conjugate adduction and asymmetric convergence, contractility in LRi did not differ significantly from LRs. During conjugate adduction, contractility in MRs was more than twice that of LRi and LRs. In comparable angles of asymmetric convergence, contractility of MRs was about one third that in conjugate adduction. This highly significant difference in compartmental behavior for the MRs confirms and extends prior MRI studies reporting differential compartmental behavior of horizontal rectus EOMs during ocular counter-rolling (Clark and Demer 2012a), and in the superior MR compartment during vertical duction (Demer and Clark 2013).

The present analysis parsed EOMs into two compartments having equal vertical dimensions corresponding to the average location of the anatomical demarcation demonstrated by MRI (Fig. 3). This conservative 50% parsing seems reasonable on average for the LR and is consistent with histological reconstructions for LR (da Silva Costa et al. 2011; Peng et al. 2010) and MR (da Silva Costa et al. 2011). However, the conservative analytic assumption is subject to effects that would all tend to diminish its sensitivity. Mis-assignment of contractility from one compartment to the other within the same EOM would reduce the statistical significance of differential compartmental function, potentially obscuring significant physiological effects, but
never creating illusory significance. The analytic method also pools the GL with the OL in each compartment. The OL contribution to contractility is not in direct series with the scleral insertion. The implications of OL contribution are uncertain, but it seems likely that OL contraction would generally reflect GL behavior, since posterior pulley shifts during static fixations are coordinated with eye rotations (Kono et al. 2002a).

Convergence Force Paradox? In the current experiment the asymmetrically converging eye adducted in association with contraction in both compartments of its MR and relaxation in both compartments of its LR. For the aligned eye during asymmetric convergence by its fellow, the pairwise average of the individually summed contractility of the MR and LR compartments was significantly negative, excluding co-contraction. This finding of co-relaxation based upon MRI morphology supports evidence of co-relaxation based upon recordings of MR and LR forces at the tendons in monkeys performing the same asymmetric convergence paradigm (Miller et al. 2002; Miller et al. 2011). The convergence “force paradox” thus remains thoroughly substantiated and extended from monkeys to humans, yet unexplained.

Miller, Davison, and Gamlin showed in monkey abducens neurons that the ratio of vergence to conjugate sensitivity is one half, implying stronger LR innervation in convergence than conjugate gaze (Miller et al. 2011). That finding seems contradictory with the current observation of similar LR relaxation in convergence and conjugate gaze. Moreover, single unit data for convergence by monkeys (Gamlin et al. 1989; Mays and Porter 1984), which predicts horizontal rectus co-contraction, also seems contradictory with force measurements of monkey MR and LR co-relaxation in convergence (Miller et al. 2002; Miller et al. 2011) that concur with the present MRI demonstration of co-relaxation in the aligned eye. However, this conundrum
may arise from the questionable final common pathway assumption about EOM structure and behavior (Miller et al. 2002).

Non-convergence Force Paradox. The mechanical organization and peripheral innervation of the horizontal rectus EOMs permits each to function as a pair of largely-independent neuromuscular compartments (da Silva Costa et al. 2011). The data illustrated in Fig. 6B indicate that hypothetical opposite contractility in the two compartments of the same horizontal rectus EOM does not cancel or offset expected force changes, since there is net corelaxation when all compartments of the MR and LR are summed during aligned convergence. Nonetheless, the data of Fig. 6 do reveal novel phenomena that deserve to be elucidated in correlation with motor neuron activity. The contractile slopes relating PPV to unconverged adduction angle in Fig. 6A are about double for MRs than for LRs, while there were no significant contractility differences for the inferior compartments. Figure 7 demonstrates greater contractility in MRs compared with any of the other three horizontal rectus compartments over essentially the full oculomotor range. Since MRI was performed during static fixation, force balance between MR and LR should be expected for every horizontal eye position, unless other EOMs or passive elastic forces contribute significantly. The observation that contractility in conjugate gaze is 100% greater in MRs and 50% greater in MRI than in corresponding LR compartments seemingly requires a major contribution of abducting force from another source, or perhaps different behavior in the OLs of the MR and LR.

Another clue is provided by the change in MR contractility during convergence, as seen in Fig. 6B. For the same range of adduction, LR contractility was not significantly different in asymmetric convergence and in conjugate adduction, while contractility in MRs in asymmetric convergence was only about one third that in conjugate adduction. Again, during static fixation,
force balance between MR and LR would always be expected, unless other EOMs or passive elastic forces contribute significantly. In fact, during convergence, Fig. 6B suggests the expected absence of significant differences in contractility between both compartments of both MR and LR. This finding implies another paradox: MRs contractility appears excessively higher than LRs contractility in non-converged gaze, although balanced during asymmetric convergence. The novel mystery, therefore, is what force might balance the excess MR force in non-converged gaze, yet disappears during convergence. Computational simulation using *Orbit 1.8* suggests that in central gaze, the net force exerted by the cyclovertical EOMs (both vertical rectus and oblique) abducts at less than 10% of MR and LR force. Not only are abducting forces of cyclovertical EOMs insufficient to compensate for excess MR contractility in conjugate adduction, but cyclovertical EOM action would require less, not more, MR force in adduction than if the cyclovertical contribution were neglected. Because monkey trochlear neurons showed more firing reduction in convergence than conjugate adduction, Mays *et al.* supposed that SO relaxation might excycloduct in convergence and, since the SO is an abductor, might additionally balance insufficient LR relaxation (Mays *et al.* 1991). While MRI during asymmetric convergence does confirm modest SO relaxation, it also shows IO contraction (Demer *et al.* 2003a). Consequently, most of the abducting force changes in the two oblique EOMs during convergence would cancel.

It has been emphasized that abducens internuclear neurons (AINs) do not transmit signals to the oculomotor neurons that are appropriate to encode position (Gamlin *et al.* 1989; Sylvestre and Cullen 2002) and velocity (Sylvestre and Cullen 2002) of the contralateral, adducting eye. This is because AINs fire like abducens motor neurons and necessarily decrease their drive to contralateral MR motor neurons in convergence when the contralateral eye adducts. Gamlin
proposed that AINs and abducens motor neurons both carry a vergence signal at about half the conjugate gain (Gamlin et al. 1989). The present MRI data roughly support this concept for MRs, as reflected in the regression slopes in Fig. 6. Inconsistent, however, is the current finding that the comparable contractility of LRi and LRs observed here was not different in asymmetric convergence and conjugate adduction.

Beyond agonist-antagonist EOM pairs, other tissues exist that mechanically load ocular rotation. Smooth muscle (SM) exists in the pulley suspensions, particularly in a band over 1 mm thick from the inferior rectus (IR) to MR pulleys (Demer et al. 1997; Kono et al. 2002b; Miller et al. 2003) and in connective tissues extending superiorly from the MR pulley towards the superior rectus (SR) pulley (Demer et al. 1997). Most SM between the MR and IR pulleys is composed of 30 – 40 micron diameter bundles arranged in an anteroposterior direction, while most between the MR and SR pulleys is arranged vertically in 10 micron diameter bundles (Kono et al. 2002b). The SM receives rich autonomic innervation (Demer et al. 1997) that could actively modulate the mechanical environment of the EOMs. Contractile SM tonus near the MR pulley would draw the MR pulley's elastic suspensions posteriorly and laterally, unloading the OL insertion to reduce passively its elastic tension, and thus reducing MR force required for adduction. Contraction of orbital SM might plausibly be reflexively linked with parasympathetically-mediated contraction of ciliary SM to reduce zonular tension mediating lens accommodation and contraction of iris sphincter SM mediating pupillary miosis. Since there is sparse SM distribution around the LR pulley, little unloading of the LR’s OL would be anticipated, consistent with the similar contractility data of Fig. 6.

The present study demonstrates differential behavior of the two MR compartments during conjugate adduction, consistent with the existence of non-overlapping motor nerve projections to
the MR compartments. The finding adds to existing data supporting differential compartmental function in the MR. In humans, MRI demonstrated highly significant contractile increases in maximum cross-section and posterior volume of MRs from infraduction to supraduction, but no significant changes for both measures in MRI, LRi, and LRs (Demer and Clark 2013).

Differential compartmental MR contractility might thus augment vertical duction. During ocular counter-rolling, MRI in the extorting orbit demonstrated significant contractile changes in LR maximum cross section and posterior partial volume in LRi but not in LRs compared with the intorting orbit (Clark and Demer 2012a). Differential compartmental contractility of horizontal rectus EOMs might thus contribute to the torsional vestibulo-ocular reflex during ocular counter-rolling.

The oculomotor nucleus is composed of subnuclei so topographically discrete that a small lesion in one can selectively impair its corresponding EOM (Castro et al. 1990; Chou and Demer 1998). The A and B groups of MR motor neurons, which receive the same premotor inputs, are widely separated by vertical rectus and IO motor neurons. There also exists a C group at the periphery (Buttner-Ennever and Akert 1981) now believed to project to palisade endings and to multiply-innervated fibers (MIFs) (Lienbacher et al. 2011). The function of the MIFs that terminate in palisade endings remains controversial, but could be sensory, motor, or perhaps both. Might the A and B groups separately innervate MRI and MRs? There currently exists no evidence concerning this intriguing possibility, but some evidence exists for topography within the motor nuclei for each EOM. Each such motor nucleus is now believed to be associated with neurons at its periphery that project to MIFs and palisade endings. All three groups of the MR subnucleus receive projections from the contralateral abducens nucleus through the medial longitudinal fasciculus (Buttner-Ennever and Akert 1981). The MR and LR motor neurons
convey both versional and vergence signals (Keller and Robinson 1972); however, despite an
early report to the contrary (Keller 1973), these motoneurons behave heterogeneously during
different ocular movements. In MR and LR motor nuclei, most cells are insensitive to vergence,
while other cells modulate both to eye position and vergence (Mays and Porter 1984). In
monkey, many abducens motor neurons correlate better during asymmetric pursuit with
contralateral than ipsilateral eye position; some have contralateral correlations exclusively (King
and Zhou 2000; Zhou and King 1996; 1998). King and Zhou supposed that the contralateral
sensitivity might represent vergence adjustments to monocular differences in mechanical loads
(King and Zhou 2000).

It is possible to surgically isolate the superior and inferior divisions exterior to the
primate LR belly, and to selectively inject these branches with different orthograde and
retrograde neural tracers (Demer et al. 2013). Preliminary Fluorogold tracing into monkey
abducens nerve branches supports anatomical reconstructions indicating non-overlapping
intramuscular distributions of the two CN6 motor nerve divisions, and further suggest that the
two LR compartments may be innervated by topographically distinct regions of the CN6 nucleus
(Demer et al. 2013). However, these preliminary findings require confirmation and extension to
the MR subnuclei.

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References


Demer and Clark MRI of Extraocular Muscles in Convergence and Adduction


### Table. Participating Subjects and MRI Image Set Acquisitions

<table>
<thead>
<tr>
<th>Study</th>
<th>Number of Subjects</th>
<th>Age of Subjects With Interpretable Data (yrs)</th>
<th>Total MRI Set Acquisitions Included in Analysis</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Subjects Participating</td>
<td>Subjects Interpretable</td>
<td>Orbits Interpretable</td>
</tr>
<tr>
<td>Asymmetric Convergence</td>
<td>20</td>
<td>11*</td>
<td>11</td>
</tr>
<tr>
<td>Matched Adduction</td>
<td>11</td>
<td>11</td>
<td>17</td>
</tr>
<tr>
<td>Full Horizontal Duction</td>
<td>15</td>
<td>15</td>
<td>25</td>
</tr>
<tr>
<td>Lateral Rectus Anatomy</td>
<td>75</td>
<td>23†</td>
<td>29</td>
</tr>
</tbody>
</table>

* - subjects able to achieve asymmetric convergence with at least one eye.
† - subjects who exhibited an anatomical feature demarcating the lateral rectus compartments.
Figure Legends

Fig. 1. Representative axial (top) and quasi-coronal T1 weighted MRI demonstrating asymmetric convergence by the left eye while the right eye remained aligned. In convergence (right column), note reduction in cross section of the relaxing left lateral rectus muscle (LR), and increase in cross section of the contracting left medial rectus (MR), as compared with distance viewing (left column). The optic nerve (ON) of the converging left eye may be seen in the upper right panel to be lateral to its position during distant target viewing.

Fig. 2. Quasi-coronal MRI of both orbits of a subject showing a fissure in the deep orbit demarcating the inferior (LRi) from superior (LRs) compartments of the LR bilaterally. Note that the fissure are not evident in the more anterior portion of the LR in the image planes 10 mm posterior to the globe-optic nerve junction (bottom), nor in any plane anterior to this location. Distances are negative representing posterior location relative to globe-optic nerve junction.

Fig. 3. Histogram showing proportionate distribution of the inferior compartment of the lateral rectus muscle as defined by anatomic demarcation visible somewhere along the muscle length by MRI in 30 orbits of 23 human adults.

Fig. 4. Mean cross sectional areas of the 50% inferior and superior compartments of the MR and LR muscles plotted for 11 subjects each in adduction and in asymmetric convergence to a 15 cm distant target with the fellow eye aligned, for contiguous 2 mm thick quasi-coronal image planes numbered positively from 0 at the globe-optic nerve junction in starting position. Note larger inferior than superior compartments for both LR and MR. Superimposed lines are best-fit Gaussian curves. For both adduction and asymmetric convergence of both LR and MR, contraction was associated with an increase and posterior shift in maximum cross sectional area.
Fig. 5. Mean locations of peaks of Gaussian fits to horizontal rectus EOM compartmental cross sections, as functions of 2 mm thick image plane number registered to zero at the plane of the globe-optic nerve junction in the reference gaze position, for both convergence and conjugate adduction. For both adduction and asymmetric convergence of both LR and MR, contraction was associated with posterior shift in Gaussian peak.

Fig. 6. Percent change in posterior partial volume (PPV) of horizontal rectus EOM inferior and superior compartments during matched adduction angles during conjugate adduction and asymmetric convergence to a 15 cm distant target with the fellow eye aligned. Linear regression slopes m ± SD represent EOM contractility. Data were obtained under conditions described in Fig. 3. Points are separately plotted for the inferior and superior 50% compartments of the MR and LR, and for both eyes. Contractility of MRs was significantly lower in asymmetric convergence than in conjugate adduction. For LR, the comparable slopes were not different than during conjugate adduction. Note the slightly negative distribution of PPV of both compartments of LR and MR of the aligned eye.

Fig. 7. Percent change in posterior partial volume (PPV) of lateral rectus inferior (LRi) and superior (LRs) compartments, and medial rectus inferior (M Ri) and superior (MRs) compartments measured for a broad range of horizontal duction in multiple subjects. For comparisons of contractility, ductions for EOM contraction (i.e. adduction for the MR and abduction for the LR) are plotted as positive. Regression slopes m ± SD indicate contractility. Contractility of LRs was slightly but significantly greater than LRi (P<0.05), but contractility of MRs was not only markedly greater than MRi, but also greater than LRs (P<0.0005 for both).
Lateral Rectus Compartment Distribution by MRI

Mean 0.49 ± 0.10 SD
N = 30

95% Confidence
Horizontal Rectus Contractility
In Conjugate Duction
(50% compartments)

- **MR Superior** $m = 1.84 \pm 0.08^{**}$
- **MR Inferior** $m = 1.27 \pm 0.06_{f}^{f}$

$\pm$ SD

** - $p < 0.0005$

Percent Change in Posterior Partial Volume

Action - deg

- **LR Superior** $m = 1.52 \pm 0.08^{**}$
- **LR Inferior** $m = 1.38 \pm 0.06_{f}^{f}$

$f - p<0.05$