

# Proprioceptive role for palisade endings in extraocular muscles: Evidence from the Jendrassik Maneuver

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## Abstract

A proprioceptive hypothesis for the control of eye movements has been recently proposed based on neuroanatomical tracing studies. It has been suggested that the non-twitch motoneurons could be involved in modulating the gain of sensory feedback from the eye muscles analogous to the gamma ( $\gamma$ ) motoneurons which control the gain of proprioceptive feedback in skeletal muscles. We conducted behavioral and psychophysical experiments to test the above hypothesis using the Jendrassik Maneuver (JM) to alter the activity of  $\gamma$  motoneurons. It was hypothesized that the JM would alter the proprioceptive feedback from the eye muscles which would result in misregistration of eye position and mislocalization of targets. In the first experiment, vergence eye movements and pointing responses were examined. Data showed that the JM affected the localization responses but not the actual eye position. Perceptual judgments were tested in the second experiment, and the results showed that targets were perceived as farther when the afferent feedback was altered by the JM. Overall, the results from the two experiments showed that eye position was perceived as more divergent with the JM, but the actual eye movements were not affected. We tested this further in Experiment 3 by examining the effect of JM on the amplitude and velocity of saccadic eye movements. As expected, there were no significant differences in saccadic parameters between the control and experimental conditions. Overall, the present study provides novel insight into the mechanism which may be involved in the use of sensory feedback from the eye muscles. Data from the first two experiments support the hypothesis that the JM alters the registered eye position, as evidenced by the localization errors. We propose that the altered eye position signal is due to the effect of the JM which changes the gain of the sensory feedback from the eye muscles, possibly via the activity of non-twitch motoneurons.

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## 1. Introduction

Knowledge of eye position is critical for accurate visuomotor behavior. For instance, to make an accurate reaching movement to pick up an object, the central nervous system (CNS) must combine several signals including the

initial hand position, head position, eye position, and retinal location of the object. The CNS can obtain eye position information from two non-visual sources: the efference copy of the motor command sent to the eye muscles (outflow) and from the eye muscle proprioceptors (inflow) (Steinbach, 1987). The debate between outflow and inflow theories goes back to Helmholtz and Sherrington (Bach-y-Rita, 1971), but during the last 20 years ample studies have provided evidence suggesting that the afferent signals from the extraocular muscles (EOM) are used during egocentric

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localization tasks (Bridgeman & Stark, 1991; Gauthier, Nommay, & Vercher, 1990; Roll, Velay, & Roll, 1991; Velay, Roll, Lennerstrand, & Roll, 1994), programming of eye movements (Knox, Weir, & Murphy, 2000; Weir & Knox, 2001), and during adaptation of smooth pursuit (Van Donkelaar, Gauthier, Blouin, & Vercher, 1997). In addition, patients show pointing errors when the proprioceptive signals from the eye muscles are disrupted, for example, after surgical interventions that destroys proprioception (Steinbach & Smith, 1981; Steinbach, Kirshner, & Arstikaitis, 1987) or due to pathology involving the trigeminal nerve (Campos, Bolzani, Schiavi, Fanti, & Cavallini, 1989; Ventre-Dominey, Dominey, & Sindou, 1996).

Although it is now recognized that proprioception contributes to registered eye position, the mechanism of proprioceptive feedback from EOM has not been established. There are two potential receptors in the human eye muscles that could provide proprioceptive information: muscle spindles and palisade endings (PEs). Muscle spindles, which are the primary proprioceptors in the skeletal muscles, have been found in the EOM of several species: human, sheep, pig, and some primates, but not in other species, such as cat, rabbit, horse, or mouse (Maier, DeSantis, & Eldred, 1974). Detailed histological studies of muscle spindles in the human eye muscles have shown that they are different from the skeletal spindles. Ruskell (1989) reported that more than 50% of EOM spindles were indistinguishable from extrafuscular fibers as they were not enclosed in a capsule and did not have a defined equatorial region. He also observed that nuclear bag fibers were virtually absent, which was also confirmed by others (Blumer et al., 1999; Lukas, Aigner, Blumer, Heinzl, & Mayr, 1994). Although the morphological differences between spindles in the EOM and those found in the skeletal muscles are well documented, the specific function of EOM spindles has not been established. Thus, it cannot be concluded at the present time whether EOM spindles can provide adequate proprioceptive signals informing the CNS about changing eye position.

Another putative source of proprioception from the eye muscles are PEs, which are receptors that are unique to EOM. PEs are associated with the multiply innervated fibers (MIFs) of the global layer and they are sometimes referred to as innervated myotendinous cylinders (Ruskell, 1978). PEs have been found in the EOMs of many species, such as cat, rhesus monkey, sheep, rat, and human (Alvarado-Mallart & Pincon-Raymond, 1979; Blumer, Lukas, Wasicky, & Mayr, 1998; Buttner-Ennever, Horn, Scherberger, & D'Ascanio, 2001; Eberhorn et al., 2005; Richmond, Johnston, Baker, & Steinbach, 1984). Anatomical studies show that the PEs are enclosed in a capsule at the distal end of the MIFs. A thinly-myelinated axon runs along the muscle fiber and then loops back to enter the capsule as it divides into several branches and makes contact with the tendon and muscle fibers (Alvarado-Mallart & Pincon-Raymond, 1979; Richmond et al., 1984).

Although the location of the cell body of PE's has not been established, several studies provide morphological

and histological evidence suggesting that PEs are among the sensory receptors which provide the CNS with proprioceptive information about eye position. Alvarado-Mallart and Pincon-Raymond (1979) reported that PEs in the cat are associated with the presence of clear vesicles which are common in other sensory endings, such as Golgi tendon organs (GTO) and muscle spindles. Billig and colleagues reported that PEs were labeled when retrograde tracers were injected into the Gasser's (trigeminal) ganglion, which contains only sensory neurons. However, recent histochemical examination of the musculotendinous junction shows that, in addition to the sensory endings, the myoneuronal region also contains motor endings (Lukas et al., 2000). These motor endings were identified based on staining of the myoneuronal junction with  $\alpha$  bungarotoxin, which labels acetylcholinergic receptors. Lukas and colleagues concluded that PEs might receive dual, sensory-motor innervation, similar to that found in the muscle spindles, which are sensory receptors innervated by  $\gamma$  motoneurons.

In line with the work of Lukas and colleagues (2000), recent anatomical tracing studies by Buttner-Ennever et al. (2001) demonstrated that the EOM receive dual innervation from two distinct groups of ocular motoneurons. The EOM of the global layer can be classified into singly and multiply innervated fibers based on the pattern of innervation they receive. The singly innervated fibers (SIFs) have a single end-plate zone located in the midregion of the muscle and respond with fast propagating action potentials when stimulated, thereby contributing to the force developed by the muscle. In contrast, the MIFs have multiple end plates distributed along the fiber which are concentrated at the distal end (this is also the region where PEs are found). Upon electrical stimulation, the MIFs respond with slow graded potentials and do not contribute to the force developed by the muscle (Fuchs & Luschei, 1971). Due to these properties, the SIFs are referred to as twitch fibers, whereas the MIFs are referred to as non-twitch fibers (Buttner-Ennever et al., 2001). When injections of horseradish peroxidase were made at the distal or the midregion of the EOM, two groups of neurons were identified. Large motoneurons were labeled when the midregion of the muscle fiber close to the end plate was injected, whereas smaller motoneurons, in a distinct region around the periphery of the large motoneurons, were labeled when the distal musculotendinous region of the muscle was injected. Based on these results, it was concluded that the large motoneurons innervate the twitch fibers (SIFs), and the smaller motoneurons innervate the non-twitch fibers (MIFs). Further work has also shown that the twitch and non-twitch motoneurons receive different pre-motor input, which sheds light on a possible role of these fibers in oculomotor control (Wasicky, Horn, & Buttner-Ennever, 2004). For instance, the twitch motoneurons receive projections from the areas within the brainstem that are involved in the programming of fast eye movements, such as saccades and the vestibuloocular reflex. The non-twitch motoneurons receive pre-motor input from areas

that are known to be involved in gaze-holding mechanisms, vergence eye movements and smooth pursuit.

The role of MIFs and PEs in the control of eye movements remains elusive. Several authors have proposed that the PEs and MIFs might have a proprioceptive role in the control of eye movements (Buttner-Ennever, Horn, Graf, & Ugolini, 2002; Porter, Baker, Ragusa, & Brueckner, 1995; Robinson, 1991). In particular, the non-twitch motoneurons of the MIF in the global layer could be involved in modulating the gain of sensory feedback from the PEs, analogous to the  $\gamma$  motoneurons which control the sensitivity of muscle spindles in skeletal muscles.

We took a behavioral approach to examine whether the gain of sensory feedback from the EOM can be altered by a manipulation that affects the activity of the  $\gamma$  motoneurons in skeletal muscles. The Jendrassik Maneuver (JM) is an isometric voluntary contraction of any muscle group. JM is referred to as a reflex reinforcing maneuver because the amplitudes of skeletal reflexes are facilitated while the JM is performed (Delwaide & Toulouse, 1981; Murthy, 1978). One of the mechanisms proposed to explain the reflex reinforcement effect is that the muscle contraction has a general effect that results in up-regulation of the  $\gamma$  motoneuron activity which increases the baseline activity of muscle spindles and, consequently, results in larger efferent response when the muscle is stretched.

Stretch reflexes have not been recorded in the EOM (Keller & Robinson, 1971); however, neural responses to EOM stretch have been recorded in several cortical regions (Donaldson, 2000). The role of proprioception in the control of eye movements is most likely different than in the control of limb position and movement but the possibility that proprioceptive feedback might be modulated by the activity of non-twitch motoneurons should not be dismissed, particularly in light of the new findings that reveal dual innervation of the EOM from the twitch and non-twitch motoneurons. We hypothesised that if the non-twitch motoneurons are analogous to the  $\gamma$  motoneurons, the JM should also change the activity of these neurons which would alter the afferent feedback from PEs and result in misregistration of eye position and pointing errors. Furthermore, if the JM affects the activity of the non-twitch motoneurons, the actual eye position should not be different between the conditions because the non-twitch motoneurons do not add to the force used to move the eyes (Fuchs & Luschei, 1971).

It has been reported that the non-twitch motoneurons receive monosynaptic input from the pre-motor centers located in caudal mesencephalic reticular formation and the supraoculomotor area, which are involved in the control of vergence eye movements (Wasicky et al., 2004). Therefore, localization responses were examined while participants performed vergence eye movements in the first two experiments. Saccadic eye movements were examined in Experiment 3 which served as a control because non-twitch motoneurons do not receive direct pre-motor input from areas involved in programming of *saccadic* eye movements.

## 2. Experiment 1

### 2.1. Methods

#### 2.1.1. Observers

Participants in all three studies had normal or corrected-to-normal visual acuity of 20/20 and stereopsis of at least 40 s of arc as measured with the Titmus test (Titmus Optical, Petersburg, Virginia 23805). All experimental protocols were approved by the Ethics Review Boards at the University of Toronto and the University Health Network. All participants gave their informed consent prior to participating. Ten healthy adults with no history of any ocular disorders, mean age  $30.8 \pm 7.2$  years, participated in the first experiment.

#### 2.1.2. Stimuli

The stimuli were two green light emitting diodes (LEDs) embedded in a custom-made black board and controlled by the experimenter via a trigger box. The stimuli were in an earth-horizontal plane and aligned with the participant's midline, slightly below eye level, and the viewing distance was 25 cm to the near target, and 45 cm to the far target.

#### 2.1.3. Apparatus

Horizontal and vertical position of both eyes was monitored and recorded using an infra-red eye-tracker system (El-Mar series 2020, Toronto, Ontario, Canada). The horizontal and vertical eye positions were obtained from the relative positions of multiple corneal reflections and center of pupil. The system accuracy is  $0.5^\circ$  with a linear visual range of  $\pm 40^\circ$  horizontally and  $\pm 30^\circ$  vertically. The system is free from drift and has a resolution of  $0.1^\circ$ . Eye position data were sampled at 120 Hz and stored on a computer for further analysis. Prior to data collection, the eye tracker was calibrated. The calibration procedure involved fixating 14 points displayed along the horizontal and vertical axes (seven fixation points along each axis), separated by  $3.3^\circ$  visual angle. The participant's head was stabilized using a chin rest and adjusted so that the eyes were in the central position when looking at the center of the array.

Arm movement data were recorded at 60 Hz using an electromagnetic device (Flock of Birds, Ascension Technology, Burlington, Vermont, USA). The resolution of the system is 0.5 mm. The receiver was placed on the participant's thumb of the dominant hand, which was used for pointing. The calibration involved passively placing the participant's thumb at the targets' location, which was performed at the end of the experimental session in order to avoid any bias or learning effect.

JM involved an isometric, voluntary muscle contraction which was performed with the abductor muscles of the legs against resistance. The device used for resistance was a Thigh Master™. Participants were asked to perform each contraction at a 75% level of their maximal voluntary contraction, which was determined prior to the initiation of the experiment. To ensure that the isometric contraction was

performed at a consistent level throughout the experiment, a string was tied around the Thigh Master™ which was pulled taut when the muscle contraction was executed. Participants were instructed to hold the string taut when performing the JM.

#### 2.1.4. Procedure

Participants were seated in total darkness with their heads stabilized by a chin rest and performed an open-loop pointing task. During the experimental procedure, participants were instructed to look and point by raising the thumb to be exactly underneath the target (green LED) as accurately as possible when cued by the experimenter. All *extraneous* visual cues were removed to ensure that participants had to use a non-visual source of information to localize the target. There were three experimental conditions randomized in five blocks of six trials as to order: (a) Control: look and point to target; (b) Task 1: look and point to the target while performing a muscle contraction (JM) with the lower limbs; (c) Task 2: look at the target while performing a muscle contraction and point 2–3 s after the contraction has been released (see Fig. 1 for illustration of the protocol).

#### 2.1.5. Data analysis

Data were analyzed using a custom software program and focused on the end-point accuracy of vergence eye movements and hand movements. Vergence angle ( $\mu$ ) was obtained by subtracting the right-horizontal eye position from the left-horizontal eye position. Vergence-specified distance ( $D$ ) was calculated using the vergence angle and the individual interocular distances ( $I$ ):  $D = I/\mu$ . Pointing error in the median plane was calculated by subtracting real target position from the hand position data.

Vergence-specified distance and pointing error data were submitted to a repeated measures, two-way analysis of variance (ANOVA) with condition (Control, Task 1, and Task 2) and target position (far, near) as the independent variables. Post hoc analysis was performed using Tukey's HSD test which was considered significant when  $p < .05$ .

## 2.2. Results

Participants systematically overshoot the target with the hand (Fig. 2) and with the eyes (i.e., converged beyond the target) in all the conditions (Fig. 3). There was a significant effect of condition [ $F(2, 18) = 11.94, p = .0005$ ]. Results from the two-way ANOVA showed no significant interaction effect between condition and target position (far or near) [ $F(2, 18) = 0.44, p < .05$ ]. Post hoc analysis revealed that pointing responses were significantly less accurate in Task 2 (mean pointing error  $6.93 \pm 5.0$  cm) compared to the control condition (mean pointing error  $5.32 \pm 5.06$  cm) and Task 1 (mean pointing error  $5.51 \pm 4.93$  cm).

There were no significant differences between the mean vergence-specified distance of any of the conditions [ $F(2, 18) = 0.26, p < .05$ ] and the interaction effect was also

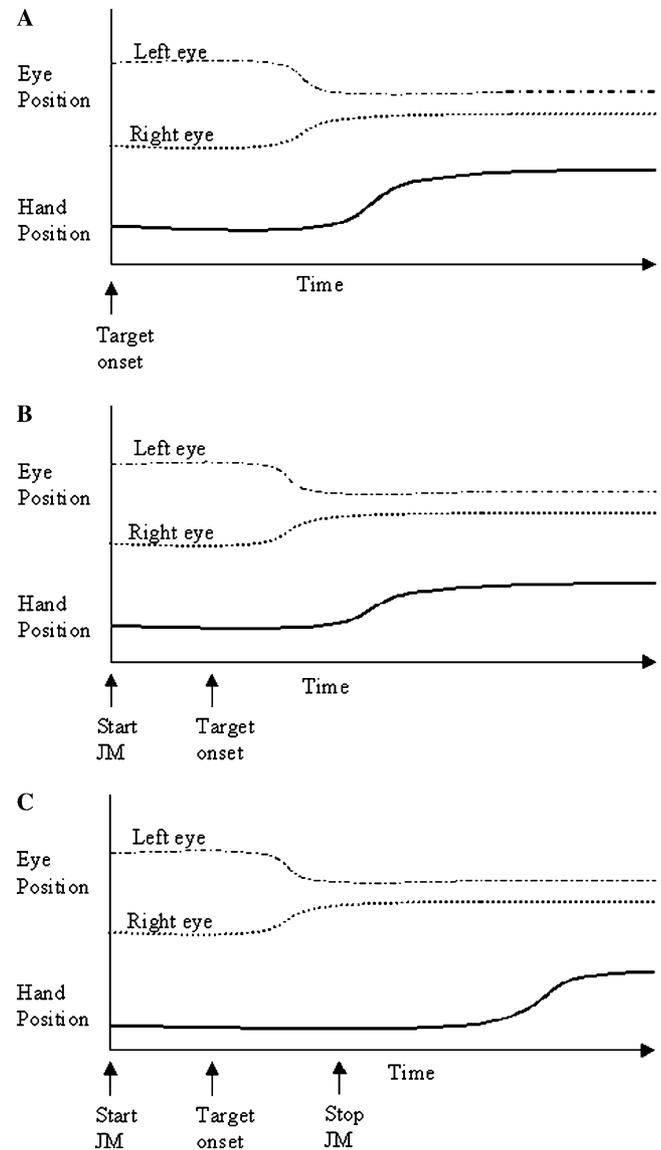


Fig. 1. Schematic illustration of the experimental protocol used in Experiment 1: (A) control task; (B) Task 1: look and point during JM; (C) Task 2: look during JM and point after JM.

non-significant [ $F(2, 18) = 1.02, p < .05$ ]. On average, participants looked beyond the target by  $62 \pm 24\%$  (mean  $\pm$  standard deviation).

## 2.3. Discussion

We hypothesized that the JM would affect the localization performance by altering the proprioceptive signal from the eye muscles, possibly via the activity of non-twitch motoneurons. Data from the study provided partial support for the hypothesis, but cannot be interpreted unambiguously. In particular, results showed that when participants first made an eye movement to the target while the JM was performed and executed the pointing response 2–3 sec after the contraction has been released, the pointing response was significantly less accurate compared to the control condition or to the task when the JM was performed through-

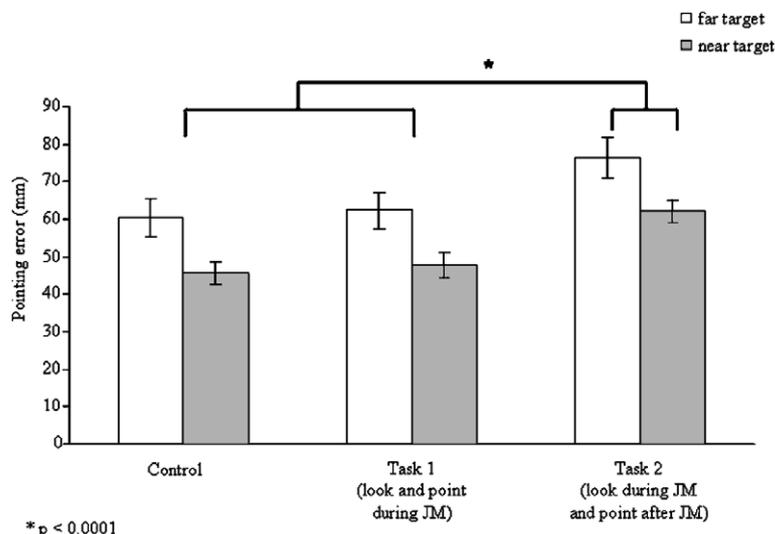


Fig. 2. Experiment 1: Mean pointing error of the hand. The figure illustrates the significant difference between Task 2 and the other two conditions (Control and Task1). Error bars show  $1 \pm$  standard error.

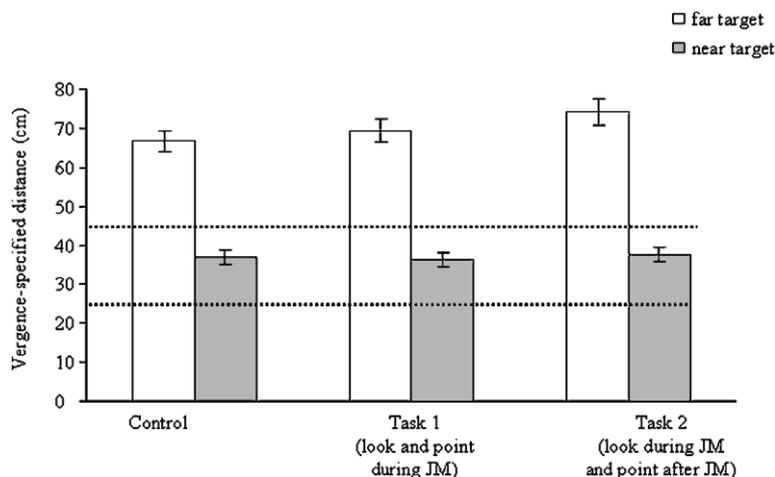


Fig. 3. Experiment 1: Average vergence-specified distance for near and far targets in all the tasks. The targets were shown at a distance of 25 and 45 cm from the participant which is shown by the dotted lines.

out the trial. It might be surprising, at first, to find no difference in pointing accuracy when JM was performed throughout the trial compared to the control condition. One possible explanation for this effect is by considering that the CNS continually monitors the afferent feedback from the EOM and that the JM alters the signal sent to the CNS. The larger pointing error was found in the condition when the eye movements and the hand movement were executed under different afferent feedback (i.e., eye movement with JM, hand movement without JM). On the other hand, no significant difference was found between the control condition and when JM was performed throughout the trial because the movements of the eyes and the hand were programmed and executed under the same afferent feedback.

Another explanation that must be considered is that the effects obtained in the present study were due to the effect of JM on  $\gamma$  motoneurons of the arm muscles used for pointing. Presumably, the JM has a general effect on all  $\gamma$  motoneurons

(Delwaide & Toulouse, 1981), and it is possible that the activity of muscle spindles in the arm muscles was also altered and might have influenced the localization response. This limitation was addressed in the next experiment.

A critical finding from this study was that the vergence eye movements and the vergence-specified distance were not affected by the JM as shown by the lack of differences between any of the conditions. These data provide support for the fact that JM does not affect the actual eye position and, consequently, the differences in localization response must be due to an altered registered eye position signal. This notion is consistent with the fact that JM should alter the proprioceptive feedback from EOM via the non-twitch neurons without altering the actual eye position because eye movements are controlled by the twitch neurons.

On average participants converged beyond the target in all the tasks, which is a finding consistent with a previous study by Malinov, Epelboim, Herst, and Steinman (2000). In that study, participants under-converged by 20–45%

while looking and tapping to targets under natural viewing conditions (i.e., head was not restrained and with full visual feedback). In the present study, participants converged even farther beyond the target, which is most likely due to methodological differences between the two studies: participants in our study had restrained head movement and no visual reference.

### 3. Experiment 2

The purpose of the second experiment was to further examine whether the eye position signal is indeed altered by the JM. The major caveat in Experiment 1 was that the JM could have affected the accuracy of the pointing response of the hand by altering the spindle activity of the arm muscles. This limitation was addressed in Experiment 2 by using an entirely visual task, which involved a criterion-free perceptual judgment task. Based on our results from the previous experiment, we hypothesized that the perceptual judgments would be significantly affected by the temporal order of the JM. In other words, it was expected that target localization would be significantly affected when one of the targets, either the first (standard target) or the second (comparison target), is shown during altered eye muscle afferent feedback.

#### 3.1. Methods

##### 3.1.1. Observers

Twenty-one healthy adults with no history of any ocular disorders, mean age  $33.4 \pm 10.6$  years, participated in the second experiment (the sample included 10 participants who also took part in Experiment 1).

##### 3.1.2. Stimuli

The stimuli were white dots (visual angle 0.24 min arc) displayed on a flat CRT monitor (refresh rate 85 Hz). The display was programmed using VPixx (VPixx Technologies, Montreal, QC), a graphics generation and psychophysics testing software, controlled by a MacIntosh G4 computer. Targets were shown in the earth-horizontal plane in the participant's midline, approximately 15.5 cm below eye level and the viewing distance for the five targets ranged between 67.6 cm and 71.7 cm. The standard target was shown at a constant location at a viewing distance of 69.7 cm. The vergence angle required to converge on the five targets ranged between 5 and 5.5°. One of the comparison targets was shown in the same location as the standard target and the other four were shown closer or farther than the standard.

##### 3.1.3. Apparatus

JM involved an isometric, voluntary muscle contraction against resistance performed with the shoulder abductor muscles (10 participants) or with the abductor muscles of the legs (11 participants). A custom-made device, based on a spring loaded scale, was used to provide resistance when

participants used the shoulder muscles to perform the JM. Participants performed the maneuver by pulling their arms apart while holding the device in their hands. The device used for resistance with the lower limbs and the JM procedure was the same as in Experiment 1.

##### 3.1.4. Procedure

Participants were seated in total darkness and performed a two-alternative forced choice task using the method of constant stimuli. At the beginning of each trial participants were instructed to look at the standard target, which was shown for 2.5 s, and to remember its location when it disappeared. The comparison target was then shown at one of five possible locations, determined randomly by the computer. Participants made a judgment by saying whether the comparison target appeared 'nearer' or 'farther' than the standard target. There were four experimental conditions: (a) Control: standard and comparison targets were shown with no JM; (b) Task 1: standard target appeared during the JM, and comparison target appeared after the JM was released; (c) Task 2: standard target appeared when the JM was not performed, and the comparison target appeared during the JM; (d) Task 3: standard and comparison targets appeared while the JM was performed (see Fig. 4 for illustration of the protocol). The experimental conditions were completely randomized. In each experimental condition, the comparison target was shown 10 times at each of the five locations for a total of 200 trials per participant.

Our prediction was that participants' judgments would be affected by the order of JM. In particular, we expected the largest difference between Task 1 and Task 2 because one of the targets, either the standard or the comparison, was presented while the feedback from EOM was altered. Task 3 served as another control condition because both targets were shown with the same, altered feedback.

##### 3.1.5. Data analysis

The proportion of 'near' responses was calculated for each participant and task at the five locations where the comparison target was shown and a psychometric function fitted. All psychometric curves were visually inspected to determine whether the type of muscle contraction (shoulder

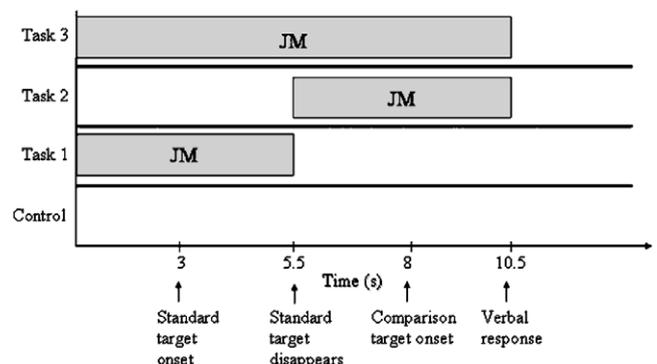


Fig. 4. Schematic representation of the experimental procedures used in Experiment 2.

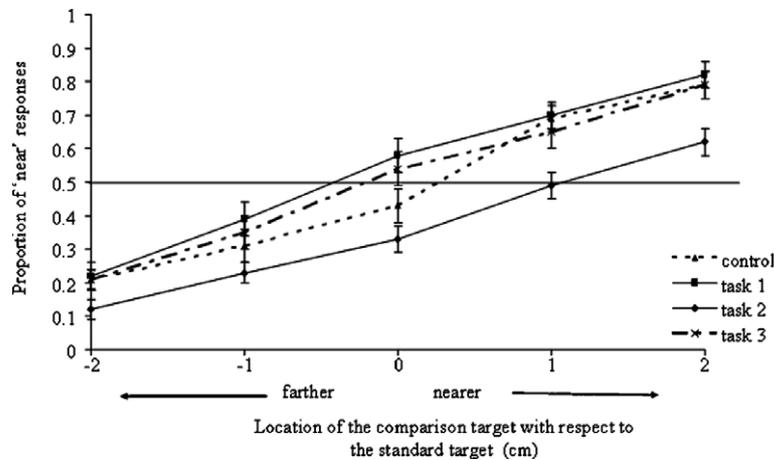


Fig. 5. Mean proportion of 'near' responses for each comparison target location (at 0 both targets were presented at the same location). Bars show  $\pm 1$  standard errors.

or leg abductor muscles) resulted in any qualitative differences. Subsequently, an overall psychometric function based on the mean of all participants was fitted for each task.

The point of objective equality (POE) was defined as the proportion of 'near' responses when the comparison target was shown at the same location as the standard target. The POEs for each participant and task were submitted to a one-way ANOVA with task (Control, Task 1, Task 2, and Task 3) as the independent variable.

Data for each participant and task was fitted using a logistic regression (SAS, ver 8.1). The goodness of fit of the model was tested using the Hosmer–Lemeshow statistic and a non-significant result was used to verify that the logistic model was appropriate. The point of subjective equality (PSE) was calculated using the estimated parameters (slope and intercept) from the logistic model. The PSE is the point at which the logistic function yields a probability of 0.5 (i.e., the comparison target is perceived as nearer than the standard target 50% of the time). Cook's distance was used to identify influential observations (outliers) in the dataset. The PSE, intercept and slope were submitted to a one-way ANOVA with task (Control, Task 1, Task 2, and Task 3) as the independent variable. Post hoc analysis was performed using Tukey's HSD test which was considered significant when  $p < .05$ .

### 3.2. Results

Preliminary inspection of the individual psychometric curves did not reveal any differences in the performance of participants who used the shoulder abductor muscles as compared to those who used the leg abductor muscles to perform the JM. Therefore, the data was collapsed and the mean performance of all participants in each condition is shown in Fig. 5. The individual data of 20 of the participants showed a consistent trend which is evident in the mean data shown in Fig. 5. Participants consistently perceived the target as farther when the JM was performed

during the presentation of the second target (Task 2). The results of one of the participants were a mirror-image of those of the rest of the group (i.e., the comparison target was perceived as nearer on Task 2), which was most likely due to a misinterpretation of the instructions. These data were not included in the statistical analysis<sup>1</sup>.

Fig. 6 shows the differences in the POE between the Control condition and Tasks 1 and 2 for individual participants (POE for Task 3 is not shown). The mean POEs across conditions were: Task 1 = 0.61, Task 2 = 0.34, Task 3 = 0.51, and Control = 0.45 [ $F(3,57) = 10.62$ ,  $p < .0001$ ]. Post hoc comparisons showed that performance was significantly different between Task 1 and Task 2. Overall, the data showed that participants perceived the location of the comparison target as nearer when the JM was performed during the presentation of the standard target (Task 1) as compared to when the JM was performed when the comparison target was shown (Task 2) or when the JM was not performed (Control).

The logistic model fitted the experimental data well for the majority of the psychometric curves (76 out of 80), which was supported by the non-significant result from the Hosmer–Lemeshow test. Although in four cases (one in the control condition and three in Task 3) the test was statistically significant, the logistic model was still used to fit the data. Two outliers were detected using the Cook's test in the PSE dataset (one observation in Task 2 and one in Task 3). These two observations were twice the magnitude of the recommended cut-off value ( $4/n$ ) and they were replaced by the geometric mean obtained from the 19 observations for a given task.

Analysis performed on the parameters obtained from the logistic regression model showed statistically significant differences between conditions for the PSE [ $F(3,57) = 13.18$ ,  $p < .0001$ ] and intercept [ $F(3,57) = 8.70$ ,  $p < .0001$ ], but not for the slope [ $F(3,57) = 0.42$ ,  $p = .7360$ ].

<sup>1</sup> Including this subject's data did not change the overall statistical results (i.e., both PSE and POE results remained significant).

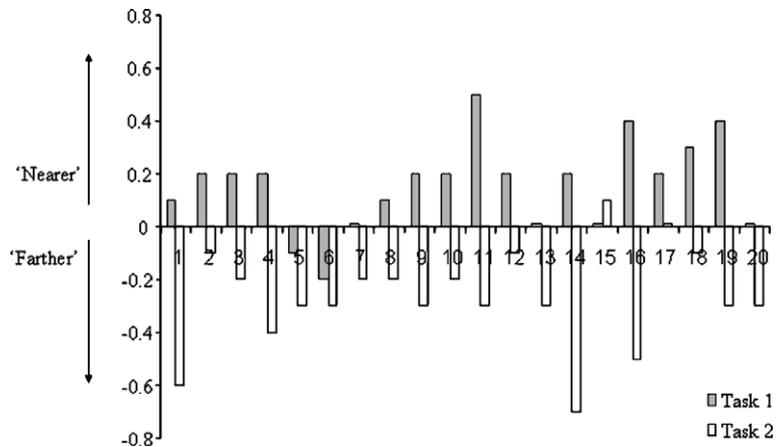


Fig. 6. Differences in the POE between the Control condition and Tasks 1 and 2 for individual participants (ID 1–20). The y-axis represents the difference in proportion of 'near' responses between Task 1 and Control and Task 2 and Control. Positive values indicate that the comparison target was reported as 'nearer' and negative values indicate that the comparison target was reported as 'farther' with respect to the control task.

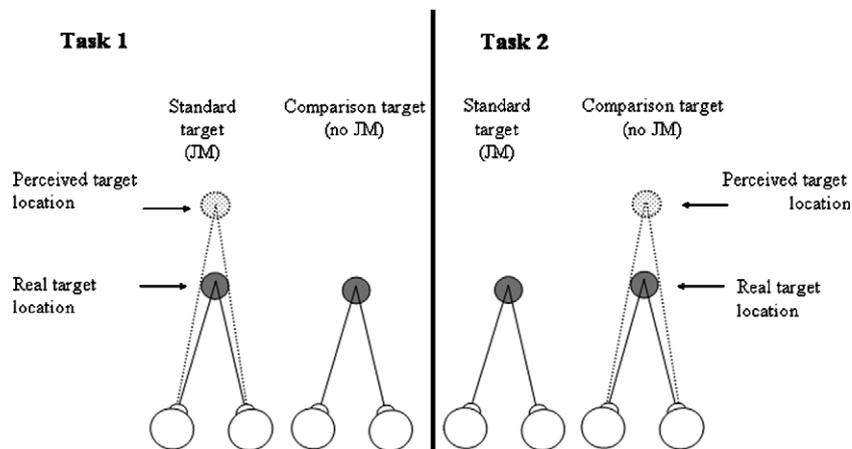


Fig. 7. Summary and interpretation of results for Experiment 2.

Post hoc analysis revealed that the PSE was significantly higher in Task 2 compared to the other conditions (Task 2 = 10.0 mm, Task 1 = 3.5 mm, Control = 5.0 mm, and Task 3 = 3.4 mm), which means that in Task 2 the comparison target had to be presented significantly nearer in order to be perceived at the same location as the standard target. The value of the intercept was significantly higher in Task 1 (0.89) than in Task 2 (0.29), the Control condition (0.61), and Task 3 (0.62). These results suggest that the JM influenced the gain but not the sensitivity of the perceptual judgments.

### 3.3. Discussion

Results from the second experiment provided support for our hypothesis that JM affects the registered position of the eyes and shed more light on the effect of the JM. A schematic diagram summarizing the results is shown in Fig. 7. In the case when both targets (standard and comparison) were shown at the same location and the JM was performed when the standard target was presented, participants reported that the comparison target was 'nearer.'

This result suggests that participants perceived the location of the standard target as farther with the JM. In contrast, when the JM was performed while the comparison target was presented, the comparison target was reported as 'farther,' which again suggests that during JM the location of the target is perceived as farther. In summary, results from the second experiment provide strong evidence that eye position is registered as more divergent when the JM is performed.

### 4. Experiment 3

The critical finding from Experiment 1 was that the JM manipulation did not affect the actual eye position. Experiment 3 was conducted to further examine whether JM has any effect on eye movements by examining different type of eye movements: the saccadic system. We chose saccadic eye movements for two reasons. First, saccades are fast eye movements programmed by different cortical and subcortical areas (Carpenter, 1988) than the vergence eye movements which were examined in Experiment 1. Secondly, a neuroanatomical tracing study has shown that the twitch

and non-twitch motoneurons receive pre-motor input from distinct brainstem areas, which are associated with the saccadic and vergence systems, respectively (Wasicky et al., 2004). Thus, we hypothesized that if the JM acts via the non-twitch motoneurons, the parameters of the saccadic eye movement should not be affected.

#### 4.1. Methods

##### 4.1.1. Observers

Ten healthy adults with no history of any ocular disorders, mean age  $32.2 \pm 12.9$  years, participated in the experiment (three participants also took part in Experiments 1 and 2).

##### 4.1.2. Stimuli

The stimulus was a white dot which subtended  $0.25^\circ$  of visual angle. The stimulus was rear-projected onto a black background and displayed at  $10^\circ$  eccentricity to the left and right of the fixation. The stimulus presentation was controlled by VPixx (VPixx Technologies, Montreal, QC), a graphics generation and psychophysics testing software, controlled by a MacIntosh G4 computer.

##### 4.1.3. Apparatus

The method of eye movement recording and the JM manipulation procedure were the same as described in the Section 2.1 of Experiment 1.

##### 4.1.4. Procedure

Participants were seated in a dimly lighted room and performed saccadic eye movements to randomly presented stimuli ( $\pm 10^\circ$  to the left and right of the fixation point). In the experimental condition, participants started the JM while looking the central fixation point and performed the JM during the saccadic eye movement. In the control condition eye movements were performed without the JM. The stimuli were shown 10 times at each location for a total of 40 trials in the control and experimental condition.

##### 4.1.5. Data analysis

Saccades that followed the presentation of the stimulus were detected using a custom software program using the velocity criterion of  $30^\circ/\text{s}$ . All saccades identified by the program were visually confirmed by the experimenter. Peak velocity and amplitude of the first saccade for each trial were determined using a custom software program. Data were submitted to repeated-measures ANOVA with condition (control, experimental) as the independent variable.

#### 4.2. Results

As expected, the data showed no significant differences for peak velocity [ $F(1,9)=0.89$ ,  $p < .05$ ] and amplitude [ $F(1,9)=0$ ,  $p < .05$ ] between the conditions. The mean peak velocity in the control and experimental conditions were  $302.61 \pm 61.97/\text{s}$  and  $306.75 \pm 63.95/\text{s}$ , respectively. The

mean amplitude of the first saccade in the control and experimental conditions were  $9.30 \pm 1.56^\circ$  and  $9.37 \pm 1.16^\circ$ , respectively.

#### 4.3. Discussion

Overall, the results from Experiment 3 suggest that the JM does not affect the actual eye movements as shown by the lack of differences in saccadic parameters between the control and experimental conditions. The negative findings from this experiment provide additional support to our hypothesis that the JM acts via the activity of non-twitch motoneurons and has no effect on the twitch motoneurons.

#### 5. General discussion

The results from the present study provide novel insight into the mechanism which may be involved in the use of sensory feedback from the EOM. Behavioral and psychophysical data support the hypothesis that the JM alters the registered eye position, but not the actual eye position. We propose that the altered eye position signal is due to the effect of the JM which changes the gain of the sensory feedback from the eye muscles, possibly via the activity of non-twitch motoneurons.

The EOM fibers can be classified into several types based on their innervation, morphological, histochemical, and contractile properties (for a review, see Spencer & Porter, 1988). Two types of efferent nerve endings are found on the EOM fibers: single, large end plates (en plaque), and multiple, small fiber endings (en grappe). Fibers that receive single innervation (SIF) have regularly spaced fibrils, large number of sarcoplasmic reticulum, and a well-developed transverse tubule system, which allows these fibers to conduct fast action potentials. In contrast, the MIF have poorly developed sarcoplasmic reticulum and do not generate action potentials, instead, they generate a prolonged graded response when stimulated at thresholds that are 3–6 times greater than the most excitable SIF (Eakins & Katz, 1972). Thus, the MIF are sometimes referred to as non-twitch fibers. Given that the MIF do not contribute to the tension developed by the muscle (Fuchs & Luschei, 1971), the question that arises is, what role could these non-twitch fibers play in oculomotor processes?

Some insight to this question comes from recent anatomical tracing studies by Buttner-Ennever and colleagues (2001) who demonstrated that the EOM receive dual innervation from separate groups of ocular motoneurons. The close association between non-twitch motoneurons, the MIF and the PEs has led several authors to propose a proprioceptive hypothesis for the control of eye movements (Buttner-Ennever & Horn, 2002; Porter et al., 1995; Robinson, 1991). In particular, more than a decade ago Robinson (1991) referred to PE and MIF as the inverted muscle spindles, and recently Buttner-Ennever and Horn (2002) suggested that the non-twitch motoneurons might have a role analogous to the  $\gamma$  efferent fibers which control the gain of

the intrafusal fibers in the skeletal muscles. The  $\gamma$  control of sensory feedback from EOM has been demonstrated in an ungulate by Whitteridge (1959). However, at the present time there is no direct anatomical evidence confirming that non-twitch motoneurons modulate the sensory feedback in primates, which is partly due to the fact that the sensory pathway and the location of the somata of the PEs have not been established. The non-twitch motoneurons share similarities with the  $\gamma$  motoneurons in that they are both smaller than their corresponding  $\alpha$  motoneurons, and their activity does not generate fast action potentials or contribute to changes in muscle tension directly. Recording from the cell body of the EOM sensory neuron while stimulating the non-twitch motoneurons would provide unequivocal evidence for a gain control regulation of proprioceptive feedback from the eye muscles.

In the present study, we used a proxy method (JM) to alter the activity of the  $\gamma$  motoneurons. While the JM is performed, the amplitude of all stretch reflexes is facilitated, which was first reported by the Hungarian physician Ernst Jendrassik (Delwaide & Toulouse, 1981). The monosynaptic tendon reflex involves only two neurons: the Ia afferent and the  $\alpha$  motoneuron, but there are several mechanisms that could be involved in the facilitation of the reflex. First, the effect could be mediated via the  $\gamma$  feedback loop: increased activity of the  $\gamma$  motoneurons would increase the gain of the muscle spindle (i.e., increased discharge rate of the spindle), which would result in a greater afferent volley when the muscle is stretched. Second, the facilitation effect could be mediated via supraspinal control which can decrease the presynaptic inhibition of the Ia afferent or increase the excitability of the  $\alpha$  motoneuron. In addition, there could be polysynaptic facilitation via interneurons in the spinal cord contributing to the effect (Dowman & Wolpaw, 1988; Gregory, Wood, & Proske, 2001; Murthy, 1978; Zehr & Stein, 1999). A detailed examination of the factors that affect the amplitude of the reflex suggested that all the above mechanisms might contribute to the reflex reinforcement effect of the JM (Delwaide & Toulouse, 1981). In particular, the contribution of the  $\gamma$  motoneurons to reflex reinforcement might be more relevant when the contraction is maintained longer than 600 ms, which was the case in the present study.

As mentioned previously, the JM has been studied extensively in the context of reflex reinforcement. Although stretch reflexes have never been recorded in the EOM (Keller & Robinson, 1971), neural activity in response to passive stretch of the EOM has been reported in cortical and sub-cortical areas (for review, see Donaldson, 2000). Clearly, the proprioceptive signals from the EOM are being used by the CNS despite the lack of reflex responses in the eye muscles.

Behavioral studies have shown that proprioceptive signals from the EOM are used during localization tasks (Bridgeman & Stark, 1991; Gauthier et al., 1990; Roll et al., 1991; Velay et al., 1994) and, as we show in the present

study, a pointing task and a perceptual judgment task. Since, presumably, JM has a general effect that up-regulates the activity of the  $\gamma$  system, we hypothesized that the eye position signal would be altered if proprioceptive feedback from the EOM is affected by the activity of non-twitch motoneurons. Our study provides preliminary support for the hypothesis. In particular, the JM affected the bias of the judgment but not its sensitivity (slope), which is consistent with the action of the  $\gamma$  motoneurons on muscle spindles (Prochazka, 1989).

In Experiments 1 and 2, participants performed vergence eye movements and the task involved judgments of absolute depth. Since all visual cues were removed, participants had to rely on the eye position signal to perform the task. A vergence task was chosen because it has been reported that the pre-motor input to the non-twitch motoneurons comes from caudal supraoculomotor area, central mesencephalic reticular formation, medial vestibular nuclei (parvocellular division), and nucleus prepositus hypoglossi (Wasicky et al., 2004), which are brainstem regions involved in vergence eye movements, ocular following, and gaze-holding mechanisms. A critical finding from Experiments 1 and 3 was that the actual eye position and saccadic parameters were not affected by the JM, which suggests that the manipulation had no effect on the  $\alpha$  motoneuron activity and did not result in change of muscle tension. Instead, the JM affected the participants' pointing and perceptual responses. Overall, these results imply that participants made judgments based on the altered registered eye position signal from EOM proprioceptors and not on the actual eye position signal which was sent to the eye muscles.

In conclusion, our results suggest that registered eye position is altered by the JM while the actual eye position is not affected. We propose that this effect may be mediated via the activity of non-twitch motoneurons. These results may have important clinical implications for the treatment of strabismus, which is an ocular disorder involving deviation of one or both eyes due to EOM imbalance. Surgical intervention, which involves cutting the EOM at the musculotendinous junction, is a common treatment for strabismus, but often does not result in regaining optimal function. Many children have to undergo multiple surgeries and yet they do not develop normal binocular function (stereoscopic vision and vergence eye movements). It is possible that the lack of success is partly due to the damage sustained at the myotendinous region of the muscles which contains the putative proprioceptors of the eye muscles (Steinbach, 1987).

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