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Change of Eye Muscle Sarcomeres According to Eye Position

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ABSTRACT

In three monkeys, the right eye was moved from the primary position to a position of 30° to 45° exotropia by suturing the globe to the orbital wall. In two animals perfused immediately following suturing, histological examination showed the medial rectus and its sarcomeres to be lengthened and the lateral rectus and its sarcomeres to be shortened as compared to those of the unoperated control eye. In the third monkey, after exotropia was maintained for 2 months, the muscle lengths changed but the sarcomere lengths were similar to those of the control eye. We interpret this to indicate addition of sarcomeres to the lengthened medial rectus and removal of sarcomeres from the shortened lateral rectus. This adaptation to conform muscle length to eye position may explain the altered eye position which persists following periods of eye deviation due to muscle paralysis, prior surgery, injury, or botulinum toxin treatment.

INTRODUCTION

Tabary et al[1] showed that holding the foot of a cat in a position of flexion resulted in internal changes of the agonist and antagonist muscles. Initially, the stretched muscle showed a lengthening of sarcomeres. Addition of sarcomeres at the ends of the stretched muscle during several weeks of maintained flexion reduced the sarcomere length to nearly normal.[2] The relaxed muscle responded over several weeks by a reduction in the number of sarcomeres. Increased stiffness and increased collagen content of the perimysium occurred along with this shortening.[3] We conducted a study in the primate extraocular muscle system to determine whether extraocular muscles adapt in this same way.

METHODS

The protocol received prior approval by the animal research committee at our institution. Three monkeys were used. Two animals had been used for other experiments, and this usage was under the anesthesia of scheduled euthanasia. With the monkeys under general anesthesia with intramuscular ketamine, 20 mg/kg, 5-0 polypropylene sutures were placed through the sclera anterior to the insertions of the superior and of the inferior rectus muscles of the right eye. These sutures were inserted through the periosteum just above and below the lateral canthus and tightened, pulling the eye into a position of 30° to 45° abduction (Fig 1). The left eye was not operated on and served as a control.

Fixation was by perfusion. A thoracotomy was made and a cannula placed into the left ventricle. Two thousand mL of warm heparinized saline were perfused by gravity, followed by 1500 mL of neutral buffered formalin at room temperature over a period of 30 minutes. Monkeys 1 and 2 were perfused within an hour of suturing. Monkey 3 was maintained in a healthy condition for 2 months after suturing. Removal of the sutures under ketamine anesthesia but before perfusion did not change the exotropic position of the right eye of monkey 3. The fixed heads were removed and placed in formalin for storage. Dissection was done by removal of the orbital bones. Individual horizontal rectus muscles were photographed in situ against a millimeter scale (Fig 2). Muscle lengths (including tendons) were measured on projected enlarged images of these photographs. Tendon lengths of the muscles of the operated eye were not different from those of the control eye and thus could not account for length changes. The muscles were then split longitudinally. From the middle of one half of each muscle, we teased individual muscle fibers or groups of a few fibers and processed them after the technique of Williams and Goldspink[4] and Muhl et al.[5]

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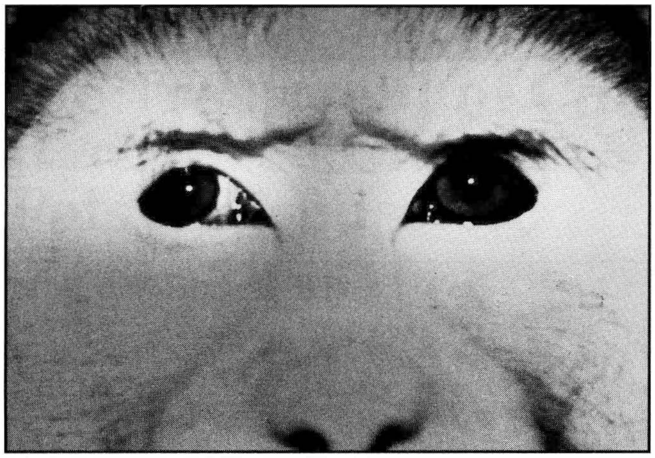

FIGURE 1: Monkey 1; after suturing.

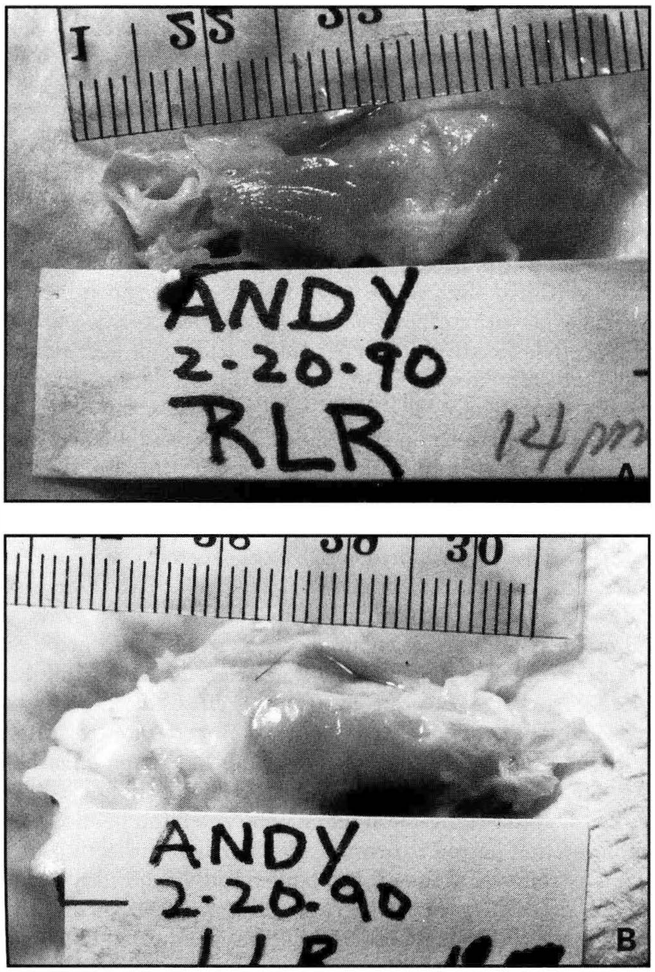

FIGURE 2: Monkey 2; lateral rectus muscle of right eye 1 hour after suturing measures 14 mm (A) compared with untreated lateral rectus muscle of left eye, 19 mm (B).

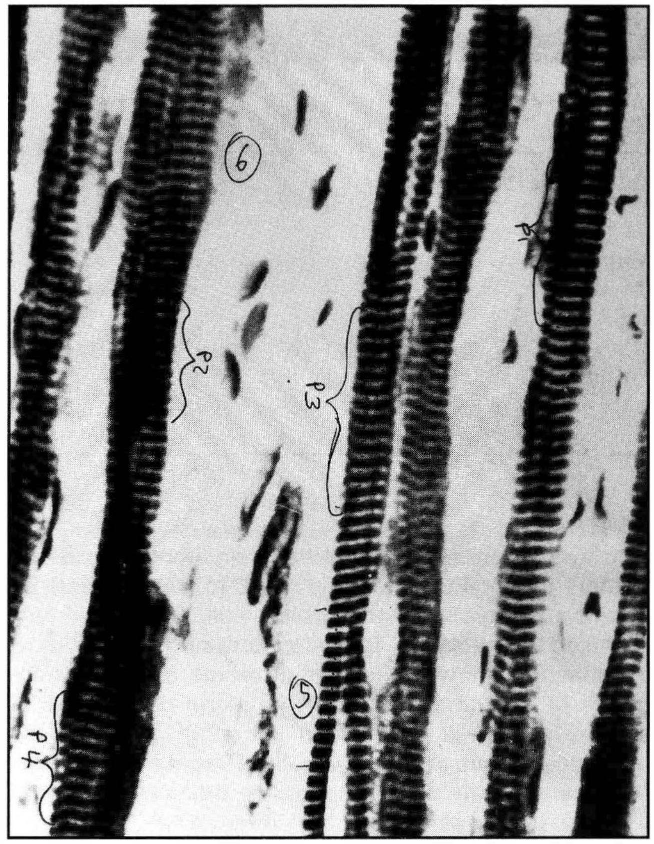

FIGURE 3: Muscle fibers showing sarcomere intervals in the right medial rectus of monkey 3, 64 days after suturing. Numbers on the Figure identify fibers used for sarcomere counts.

Paraffin tissue blocks were also made from the middle third of each muscle and longitudinal sections from these were stained with hematoxylin and eosin. An occasional fiber may have come from the orbital layer, but most were from the global layer of the muscles. Where at least eight sarcomeres in a row could be identified in a single fiber from magnified photographs of the teased fibers or section, the sarcomere intervals were measured (Fig 3). A photograph of a standard reference micrometer slide taken with the same microscope and camera and enlarged in the same way as were the muscle fiber photographs was used to calibrate a digitizing pad. The muscle photographs were placed over the pad. For each muscle fiber used, a pressure mark was made at each Z line. The underlying digitizing pad recorded these. The computer took the difference from one Z line to the next as a sarcomere interval. For each fiber measured, the data were displayed to be sure there was not a mistake such as missing one Z line resulting in an interval twice the real value. When such an outlier occurred, the fiber was remeasured before entering the data. All measured intervals were averaged for each muscle. The standard deviation is that of the pooled sample for each muscle.

RESULTS

The data of this experiment are presented in the Table. Column 1 shows the muscles measured and column 2 shows

TABLE
Comparison of Sarcomeres in Treated (Right) and Untreated (Left) Eyes of Three Monkeys

1	2		3		4	
	Sample		Muscle Length Millimeters	Difference	Sarcomere Length	
Monkey No. and Muscle	No. of Fibers	No. of Sarcomeres	RE	Versus LE	Microns ± SD	Difference RE Versus LE
Monkey #1 Perfused Day of Suturing						
RMR	37	50	20	+18%	2.73 ± .31	+16% $t(1386) = 25.8$, $P < .001$
LMR	52	887	17		2.36 ± .22	
RLR	40	677	14	−30%	1.88 ± .22	−21% $t(1204) = 34.0$, $P < .001$
LLR	38	529	20		2.37 ± .28	
Monkey #2 Perfused Day of Suturing						
RMR	53	498	20	+33%	2.91 ± .33	+26% $t(1308) = 33.9$, $P < .001$
LMR	65	812	15		2.31 ± .30	
RLR	51	681	14	−26%	1.88 ± .22	−21% $t(1183) = 37.7$, $P < .001$
LLR	40	504	19		2.38 ± .25	
Monkey #3 Perfused 64 Days After Suturing						
RMR	58	699	20	+25%	2.26 ± .23	+1% $t(1443) = 2.9$, $P = .004$
LMR	50	746	16		2.23 ± .22	
RLR	54	654	16	−25%	2.38 ± .25	+8% $t(1606) = 14.4$, $P < .001$
LLR	50	954	20		2.20 ± .23	

RE = right eye, LE = left eye, SD = standard deviation.
Muscles: RMR = right medial rectus, LMR = left medial rectus, RLR = right lateral rectus, LLR = left lateral rectus.

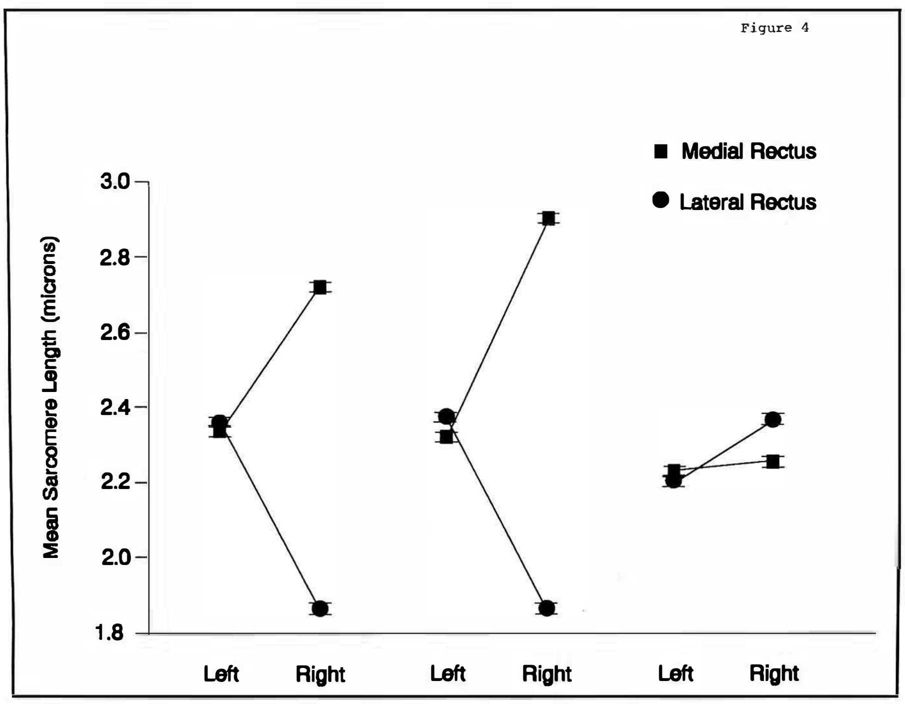

FIGURE 4: Comparison of average sarcomere length (± standard error) lateral rectus and medial rectus muscles in the right (treated) eye and the left (control) eye of monkeys 1, 2, and 3. (Left = left eye; Right = right eye.)

the large sample size of fibers measured for sarcomere interval. Column 3 confirms that, for each of the three monkeys, muscle length was altered in the expected way by surgery. In particular: the medial rectus muscle in the right (exotropic) eye was longer than in the left (control) eye by 18%, 33%, and 25% in monkeys 1, 2, and 3, respectively.

The Table's column 4 and Figure 4 show that for monkeys 1 and 2 (immediately perfused), the medial rectus sarcomeres were longer and the lateral rectus sarcomeres were shorter in the right (exotropic) eye than in the left (control) eye. Unpaired t-tests confirmed that these differences between the right eye and the left eye were statistically significant.

Figure 4 shows that for monkey 3 (perfused at 64 days after suturing), the medial rectus sarcomeres were nearly the same length in both eyes. Unpaired t-tests (Table, column 4) revealed that although differences between eyes were small, they were statistically significant. The medial rectus sarcomeres were slightly longer in the right eye than in the left eye (though not nearly as long as the medial rectus sarcomeres in the right eyes of monkeys 1 and 2). The lateral rectus sarcomeres were actually longer in the right eye than in the left eye.

Figure 4 shows that the effects of suturing were much greater in monkeys 1 and 2 (immediately perfused) than in monkey 3 (perfused 64 days after suturing). This was verified in a three-way between-subjects analysis of variance (2 muscles × 3 monkeys × 2 eyes). In particular, the differences between eyes were greater in monkeys 1 and 2 than in monkey 3: $F = 1175$, $P < .0001$.

The results were not due to statistical outliers or any need to transform the data. The data were appropriate for standard analyses of variance (ie, t- and F-tests). Prior to the statistical analyses, we examined visually the sarcomere length distribution histograms for each muscle and found that each conformed to a normal (bell-shaped) distribution. There were no obvious outliers in any histogram, nor were any of the distributions skewed significantly in any direction. All conditions had roughly the same amount of variance; standard deviations were roughly equal (Table, column 4).

DISCUSSION

Eye muscles typically lengthen and shorten 25% to 33% over the range of eye movement, so the acute changes in muscle length and sarcomere length in monkeys 1 and 2 are expected. The change in sarcomere interval over time after suturing is a result comparable to the classic studies of Goldspink et al[6] and Williams and Goldspink[2] showing adaptation of muscle length to changed limb position in cats by addition or removal of sarcomeres. In their experience, the sarcomere changes began in a few days, the method was sensitive to about 10% length change, and the sarcomere changes were reversible when limb position and movement were restored. These changes also occurred when the muscle was denervated, as might occur in paralytic strabismus or after botulinum injection. We did not feel justified in increasing the use of animals (primates or others) to the numbers which would be required to independently determine the minimum and maximum amounts of induced strabismus and minimum and maximum time duration or rates at various times for this sarcomere adaptation to occur. Since the general response we observed was similar to cat limb muscle, we assume the eye muscle responses in time and amount are parallel to the limb muscle responses in the reports cited above. The variability (standard error) in sarcomere lengths in the exotropic right eye muscles of monkey 3 (64 days) was not different from that of the control left eye muscles. This is compatible with the idea that all types of muscle fibers within the central half of the global part of the muscle had a similar sarcomere change; differences in response between the different muscle fiber types might have created a bimodal or wider distribution of responses in the treated muscles.

The clinical finding termed "contracture," a shortening and increased stiffness developing in eye muscles opposed by a paralyzed antagonist or shortened by surgical recession, seems likely to be a result of this mechanism. The rapid (7-day) stiffness reduction and reduced eye deviation following botulinum injection of such contracted antagonist muscles in paralytic strabismus[7] indicate that these changes may reverse very quickly. Simonsz has proposed that these adaptations may also serve to explain surgical outcomes in some comitant strabismus cases.[8]

REFERENCES

1. Tabary JC, Tabary C, Tardieu C, Tardieu G, Goldspink G. Physiological and structural changes in the cat's soleus muscle due to immobilization at different lengths by plaster casts. *J Physiol.* 1972;224:231-244.
2. Williams PE, Goldspink G. Longitudinal growth of striated muscle fibers. *J Cell Sci.* 1971;9:751-767.
3. Williams PE, Goldspink G. Connective tissue changes in immobilised muscle. *J Anat.* 1984;138:343-350.
4. Williams PE, Goldspink G. Changes in sarcomere length and physiological properties in immobilized muscle. *J Anat.* 1978;127:459-468.
5. Muhl ZF, Grimm AF, Glick P. Technique for measurement of sarcomere length of striated muscle. *J Dent Res.* 1976;55:170.
6. Goldspink G, Tabary C, Tabary JC, Tardieu C, Tardieu G. Effect of denervation on the adaptation of sarcomere number and muscle extensibility to the functional length of the muscle. *J Physiol.* 1974;236:733-742.
7. Scott AB, Kraft S. Botulinum toxin injection in the management of lateral rectus paresis. *Ophthalmology.* 1985;92:676-683.
8. Simonsz HJ. *The Mechanics of Squint Surgery.* Habilitationsschrift, Justus Liebig Universitat, Giessen; 1989:96.