this author a higher stimulus intensities. This implies that the CNR is present across the visible spectrum in both species tested, but differs in the relative ease of elicitation (and presumably in relative visual significance).

Despite these precautions in interpretation, these spectral response curves are sufficiently different from paradigmatic expectations to be considered significant variations (and elaborations) of the basic vertebrate visual response patterns.

a) Neither set of curves shows a Purkinje shift upon light adaptation, a property common to most retinal potentials. This implies that the CNR does not originate from the proximal retina, but from more distal cell layers.

b) The monophasic *Cichlasoma* peak and the triphasic *Hemichromis* peaks correspond with known or suspected areas of vertebrate cone activity. They match well with cyanopsin, iodopsin, and Dartnall's[2] pigment 467.

c) The latency of the CNR (circa 80 msec) is longer than that of the b-wave and the proximal negative response. Its origin by this criterion would be expected to be in the more distal retina, possibly involving interactions at the amacrine cell layer. The occasional appearance of spike bursts (2–4 spikes) at the peak of the response supports this inference, since only amacrine and ganglion cells spike.

d) The spectral responses correspond well with the expression of nuptial colors in the species tested, although the *Hemichromis* red sensitivity is not monophasic. The persistence of the blue peak in *Hemichromis* may indicate that the blue sensitivity via this response is common to the cichlid family, and that the enhancement of other spectral sensitivities might be a more limited phenomenon.

The occurrence and spectral distributions of the CNR in these cichlid species, coupled with their well-documented dependence upon color cues in territorial and reproductive behaviors (Baerends and Baerends van Roon)[3], demonstrate an additional information processing and wavelength discrimination capability in the vertebrate retina. The behaviors of these fishes also indicate that these sources of retinal information may be primary determinants of visual orientation responses critical to the maintenance of the species.

1 D.M. Bell, Experientia *35*, 342 (1979).
2 H.J. Dartnall, J. Physiol. *116*, 257 (1952).
3 G.P. Baerends and J.M. Baerends Van-Roon, Behavior, suppl. 1 (1950).

Does tenotomy of skeletal muscle alter Z-line width in older animals?[1]

Dorothy Chase, W.C. Ullrick, K.A. Dasse, D.J. Burke and A.H. Goldberg

Boston University School of Medicine, 80 East Concord Street, Boston (MA 02118, USA), 27 April 1981

Summary. Results of unilateral achilles tenotomy on older (350–410 g) male rats, showed that the general width of Z-lines in the tenotomized muscles was not significantly altered by this procedure. Streaming of the Z-lines and rod formations was still present, as in younger rats.

A variety of pathological conditions[2,3] as well as tenotomy[4,5] are known to alter the morphology of vertebrate skeletal muscle Z-lines. The changes more frequently cited are Z-line streaming and rod formation. A few years ago this laboratory reported that tenotomy caused a generalized widening (+15%) in all the Z-lines of the affected muscles, a study done in young (150–300 g) growing rats[6].

It has been suggested that the Z-line changes which occur in tenotomy represent a proliferation of Z-material as a step in the development of new sarcomeres[7–9], presumably in response to a sudden loss of tension in the muscle. The question immediately arises then: would the Z-line changes still be present if the tenotomy procedure was carried out in older animals with very slow or zero growth rates? The following study was designed to answer this question.

Materials and methods. Achilles tenotomy, including removal of a 2–3 mm segment of tendon, was performed on 1 leg of 350–410-g male albino rats, with the unoperated leg serving as the control. The animals were sacrificed by decapitation 1, 2, and 3 weeks after tenotomy, and the soleus muscles excised and placed in ice cold buffer (0.1 M KCl, 1 mM $MgCl_2$, 5 mM EGTA, 5 mM sodium pyrophosphate, pH 6.8).

Under the dissecting microscope, fibers were dissected free, tied to 3-cm fragments of wooden applicator sticks at approximately rest length, and then placed at 4 °C in 4% glutaraldehyde in buffer (7.5×10^{-2} M KCl, 7.5×10^{-4} M $MgCl_2$, 7.5×10^{-3} M Na_2HPO_4, 7.5 ± 10^{-3} M KH_2PO_4, pH 7.0). The samples were then post-fixed in 1% OsO_4 for 1 h, dehydrated in a graded series of ethanol and embedded in a mixture of araldite 502 and dodecenyl succinic anhydride. Longitudinal thin sections were cut at 60–70 nm on a Porter-Blum MT 2 microtome, and mounted on 200-mesh copper grids. The sections were then stained with 1% PTA, 10% uranyl acetate, and Reynold's lead citrate and examined with a Siemens Elmiskop I electron microscope.

Effect of tenotomy on soleus muscle Z-line, A-band width, and sarcomere length in older rats

Paired muscles*	Duration of tenotomy (weeks)	Sarcomere length (μm)**	A-band width (μm)**	Z-band width (nm)**
C^{68}	1	2.33	1.13	100.58
E^{68}	1	2.03	1.22	102.92
C^{69}	1	1.77	1.12	109.94
E^{69}	1	1.80	1.13	107.60
C^{72}	2	1.98	1.08	91.23
E^{72}	2	1.69	1.30	102.14
C^{73}	2	1.93	1.05	115.40
E^{73}	2	1.61	1.15	110.72
C^{74}	3	1.99	1.17	128.65
E^{74}	3	1.82	1.07	102.14
C^{75}	3	1.96	1.10	106.82
E^{75}	3	1.75	1.10	107.60
Mean difference		0.21	0.05	3.25
SD of mean difference		0.13	0.10	12.58
p-value, paired comp.		< 0.05	> 0.05	> 0.05

* Specimens labelled 'C' represent control muscle; 'E' contralateral tenotomized muscle. ** Values represent means of measurement within each muscle.

Electron micrographs were taken at the same magnification of both the control and experimental soleus muscles, care being taken to avoid areas in the tenotomized muscle that abounded with rod shaped or streaming Z-structures.

Direct measurements were taken (independently, by 3 of the authors) from 20×25 cm enlargements of the EM plates, using a metric ruler for sarcomere and A band length, and a desk-top magnifier containing a reticle with 0.1-mm gradations for Z-widths. As Z-lines tend to be very irregular across the width of the myofibril, measurements were taken at multiple intervals along each Z-line in a micrograph. A total of 1641 control Z-line widths, and 1735 Z-line widths in tenotomized muscle were measured; the data are contained in the table.

Results and discussion. The table presents the results of our analysis of paired muscles from 6 tenotomized animals. The mean Z-disc width of the control contralateral leg was 109 nm, the same as in the younger series of rats on which we previously reported[6]. Those of the tenotomized leg averaged 106 nm, ranging from 102 to 111 nm, and there was no significant difference between the control and tenotomized legs. In contrast to younger rats, we conclude that tenotomy is without effect on the general Z-line widths of older rats. As in the former experiment, however, occasional Z-line streaming and rod formation was observed in the experimental muscles. This has also been reported in aged rats by Fukisawa[10].

Note from the table that although there was no difference in the width of the control and experimental A-band, the sarcomere length of the experimental leg (mean = 1.78 μm) was significantly shorter than the control (mean = 1.99 μm). This of course is a consequence of tenotomy, but it might suggest that had we measured the Z widths only in those sarcomeres of identical length, the experimental Z-band width would have perhaps been wider. However, we have not found any statistical correlation between Z-disc width and sarcomere length in any group of experimental or control animals. Z-line width, in vertebrate muscle, is not a function of sarcomere length. We hold therefore with the conclusion that tenotomy does not produce a generalized widening of the Z-line in older rats. This may, in some way, be related to the flat growth curves of these animals. The finding that rod formation and streaming are still present, suggests that the stimulus or underlying cause for generalized widening may be different from that or those responsible for the other morphological changes.

1 This investigation was supported in part by NIH grants 1-RO1-NS-14033 and 1-T32-07224.
2 A.G. Engel, in: Exploratory Concepts in Muscular Dystrophy and Related Disorders, p.398. Excerpta Medica Foundation, Amsterdam 1967.
3 H.Y. Meltzer, R.W. Kunel, J. Click and U. Yang, Neurology *26*, 853 (1976).
4 S.A. Shafig, M.A. Gorycki, S.A. Asiedu and A.T. Milhorat, Archs Neurol. Chicago *20*, 625 (1969).
5 J.S. Resnick, W.K. Engel and P.G. Nelson, Neurology *18*, 737 (1968).
6 D. Chase and W.C. Ullrick, Experientia *33*, 1177 (1977).
7 S.P. Bishop and C.R. Cole, Lab. Invest. *20*, 219 (1969).
8 J. Auber, J. Microsc. *8*, 197 (1969).
9 M.J. Legato, J. molec. Cell Cardiol. *1*, 425 (1970).
10 K. Fukisawa, J. neurol. Sci. *24*, 447 (1975).

α-Tocopherol reduces fluorescent age pigment levels in heart and brain of young mice

Patricia Kruk and Hildegard E. Enesco

Department of Biology, Sir George Williams Campus, Concordia University, Montreal (Québec H3G 1M8, Canada), 10 February 1981

Summary. The levels of fluorometrically measured lipofuscin, or age pigment, were significantly lower in the brain and the heart of α-tocopherol (vitamin E)-injected mice as compared to untreated control mice at 3 and at 5 months of age.

Lipofuscin, or fluorescent aging pigment, accumulates progressively with age in mammalian post-mitotic tissues, especially in brain and heart[1,2]. It is present even in young animals[3]. Tappel et al.[4] have demonstrated that lipofuscin accumulation is reduced by feeding antioxidants, including vitamin E, to 9-month-old mice for periods ranging from 0.6 to 1.2 years.

The questions which we asked in the present study were as follows: a) Can we alter lipofuscin accumulation in young mice at 3–5 months of age by α-tocopherol (vitamin E) administration? b) Can we alter lipofuscin levels if the α-tocopherol is administered by i.p. injections? c) Will the α-tocopherol have any effect on lipofuscin accumulation over relatively short periods in young animals?

Table 1. Data for heart. The level of lipid peroxidation fluorescence product in 0.2 g heart tissue is presented for mice injected with 1.1 IU α-tocopherol, as compared to control mice at 3 and 5 months of age. The standard deviation of the measurements is presented. The level of significance as determined by the t-test is presented as a function of age and as a function of treatment. N refers to number of measurements

Age	N	Fluorescence units (±SD) α-Tocopherol	Control	Difference (%)	Level of significance
3 months	3	43.00±2.82	53.00±5.10	23%	$0.10 < p < 0.05$
5 months	7	46.93±2.04	71.79±1.58	53%	$p < 0.001$
Level of significance		NS	$p < 0.001$		

Table 2. Data for brain. The level of lipid peroxidation fluorescence product in 0.2 g brain tissue is presented for mice injected with 1.1 IU α-tocopherol, as compared to control mice at 3 and 5 months of age. The standard deviation of the measurements is presented. The level of significance as determined by the t-test is presented as a function of age and as a function of treatment. N refers to number of measurements

Age	N	Fluorescence units (±SD) α-Tocopherol	Control	Difference (%)	Level of significance
3 months	6	47.75±3.29	56.75±3.51	19%	$p < 0.005$
5 months	14	47.18±4.13	73.64±5.43	56%	$p < 0.0001$
Level of significance		NS	$p < 0.001$		