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Differential elasticity of the immature retina: A contribution to the development of the area centralis?

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Abstract

Differential stretch of a retinal surface with an initially uniform cell density has been repeatedly implicated as one of the developmental mechanisms that produces the topographic organization of cell density in the adult retina, notably the area centralis or visual streak *versus* peripheral regions. It is known that intraocular pressure is required to produce the normal conformation and thinning of the retina during development. We tested the possibility that the retina has elastic properties that might permit differential stretch in conjunction with intraocular pressure. The relative deformation of the retina containing the presumptive area centralis was compared to the deformation of peripheral retina at equivalent applied fluid displacements in 7–12-day-old cats. The peripheral retina deformed significantly more, consistent with the hypothesis that differences in the local elasticities of the developing neural retina contribute to its characteristic topographic changes. Thus, a biomechanical property of the growing eye may contribute to the mechanism by which the pattern of the visual array is sampled.

Keywords: Retinal specializations, Area centralis, Visual streak, Retinal stretch, Retinal Growth, Development

Introduction

High-acuity vision in vertebrates is subserved by specialized retinal areas which contain elevated densities of ganglion cells, photoreceptors, and the other neural retinal elements which are organized into such species-characteristic structures as the area centralis of the cat, the visual streak of the rabbit, or the fovea in primates. The mechanisms which produce these species-typical retinal specializations have recently received much attention, and retinal development appears to make use of a variety of factors including the differential generation of cells, the selective death of cells, and growth of the retina to produce these features (Stone et al., 1982; Mastronarde et al., 1984; Lia et al., 1987; Sengelaub et al., 1986).

Greater increase in area of the retinal periphery compared to the retinal center during development, resulting in a relative thinning of the density of ganglion cells, receptors, and other neural elements in the peripheral retina, has been demonstrated in a number of mammalian retinas, including the cat (Stone et al., 1982; Lia et al., 1987; Mastronarde et al., 1984), gerbil (Wikler, 1987), hamster (Sengelaub et al., 1986), rabbit (Robinson et al., 1986), and ferret (Henderson et al., 1988). This process begins early in retinogenesis as soon as the layers of the neural retina can be recognized (Robinson, 1987) and continues postnatally into the period of early visual function (Mastronarde et al., 1984). The mechanism of peripheral thinning is not known, although there are several plausible hypotheses. Cellular processes might actively grow more quickly in the peripheral retina, or a conformational change of the entire retina ("flattening") could passively alter the spacing of elements (Mastronarde et al., 1984). Alternatively or additionally, because intraocular pressure is required for the normal growth of the eye and overall thinning of the neural retina (Coulombre & Coulombre, 1956), the retina might resemble a balloon with areas of greater or lesser elasticity, resulting in greater stretch of the more elastic areas when inflated (Sengelaub et al., 1986; Robinson, 1987).

In the present experiment, we have measured the relative deformation of peripheral and central areas of the cat retina during a period of development in which differential rates of expansion of the retinal periphery and center have been documented (Mastronarde et al., 1984). We therefore tested the plausibility of models of early retinal development that de-

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pended upon differential elasticity of the growing retina as a means of producing specialized retinal topography (Mastronarde et al., 1984; Robinson, 1987; Sengelaub et al., 1986).

Methods

Subjects were six kittens, between 1 and 2 weeks of postnatal age (n = 1,7 d; n = 2, 10 d; n = 1,11 d; n = 2,12 d) from three litters. This age was chosen because at this time the full cell complement of the optic nerve axons has stabilized (Ng & Stone, 1982), but the retina has still to undergo substantial growth, conformational change and elaboration of its area centralis, and visual streak. For example, the equivalent diameter of the retina at this age is between 60 and 70% of the adult value (Mastronarde et al., 1984).

To obtain measures of retinal deformability, retinal segments (see below) were mounted on a small capillary tube (outer diameter = 1.18 mm) which was prefilled with saline, and included a small air bubble (which filled the diameter of the tube) to measure fluid excursion. The capillary tube was open at the end opposite the retinal segment. This small capillary was then inserted into a larger capillary tube (inner diameter = 1.24 mm; thus 60 μm total clearance between the two tubes), trapping the retinal segment and establishing a seal between the two capillary tubes. Retinal thickness at this age for both central and peripheral retina is in excess of 150 μm with the central retina somewhat thicker (interpolated from Robinson, 1987 and Rodieck, 1973), so the retina between the tube walls was quite compressed. Adherence of the tissue to the glass at the edges of the insertion point of the smaller capillary tube appeared to produce the effective seal. This procedure rendered both the central and peripheral retinal segments flat across the mouth of the inner capillary. Because the retinal thickness of both retinal center and periphery was so much in excess of the capillary tube tolerance, we view it as unlikely that the insertion procedures made the retinas differentially taut, although we cannot rule out this possibility from direct measurement.

The larger tube was connected to a 5-μl syringe (Hamilton) by a short length of semirigid polyethylene tubing. Total fluid volume of the closed system was 100 μl (see Fig. 1A). Starting conditions were at atmospheric pressure. Pressure changes effective to deform the retina in this system were extremely small, 6×10^{-7} mm Hg, consistent with a 0.2% volume change in a system with semielastic components. These were measured with a manometer at volume changes giving pressure changes of 1-10 mm Hg and extrapolated, employing the linear relationship of pressure and volume.

Animals were given a lethal dose of Nembutal, perfused with physiological saline, and the eyes dissected out and measured with calipers along their anterior-posterior, superior-inferior, and nasal-temporal axes. The eyes at this age were quite spherical with the mean diameters along the major axes measuring 8.64 ± 0.86 mm, anterior-posterior; 8.71 ± 0.83 mm, superior-inferior; and 9.09 ± 0.94, nasal-temporal. The neural retina was then dissected free of overlying sclera, cornea, and choroid, and the lens and vitreous humor removed. Except for orienting marks made at the superior margin of the retina and the horizontal meridian, the retina was not otherwise cut or manipulated: specifically, it was not flat-mounted to avoid possible interaction of retinal elasticity with the topological problem of mounting a hemisphere on a flat surface. With

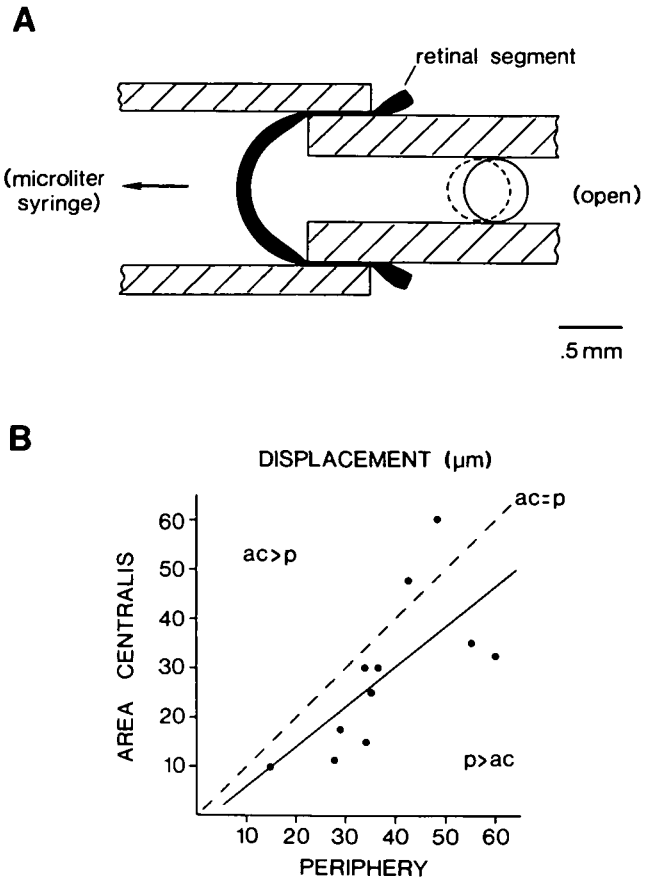

Fig. 1. A, Schematic of apparatus used to measure retinal elasticity. Retinal segments created a seal between two saline-filled capillary tubes, and a microliter syringe attached to one end of the tubing was used to induce retinal deformation by fluid withdrawal. Solid and dashed circles indicate position of the air bubble used to gauge fluid displacement induced by retinal deformation. B, Fluid displacement for retinal segments containing the presumptive area centralis plotted against the mean of the two peripheral segments for the same eye. If retinal areas were equally elastic, all points would fall on the dashed line (ac = p). However, greater elasticity of peripheral retinal segments (p > ac) results in a clustering of points below the ac = p line. The solid line is the regression line through these points ($y = 0.818x - 2.42$) which is significant [$F = 8.01$ (8,10) $P < 0.025$].

the retina floating in a saline bath, 2 × 2 mm segments of retina were cut from midtemporal retina on the horizontal meridian, and one each from the superior and inferior margins of the nasal retina. The area dissected out to include the area centralis exceeds the dimensions of the area centralis proper. Because the entire midtemporal area is elevated in cell density relative to the peripheral samples, the "area centralis" sample is a tissue area with high cell density that includes the area centralis, not the area centralis alone. The retinal segment was carefully draped on the small capillary tube as shown in Fig. 1A, with the vitreal surface of the retina toward the capillary tube, which was then inserted as described above into the apparatus.

Two measures were taken to determine the relative deformability of a particular retinal segment. First, to determine the total deformation the retina would permit, the syringe was carefully withdrawn while the retina was observed through a dissecting microscope at 15×. Retinal punctures or leaks could be

easily seen by particulate movement through the retina, and if observed, the retinal segment was discarded. This method therefore measures the ability of a retinal segment to resist deformation induced by a partial vacuum. As the syringe was withdrawn, the retina became increasingly convex until reaching a point at which its shape change ceased. Continued withdrawal beyond this point would cause the retina to detach, which was avoided. The volume of fluid withdrawn, at which increasing retinal convexity ceased, was measured five times.

A second measure of retinal elasticity was made by observing the movement of the air bubble included in the small capillary tube after a known fluid displacement (0.2 μl). This standard fluid displacement produced movement of the retinal segment, which in turn induced a displacement of fluid (and the included air bubble) in the open capillary. Bubble movement after the standard fluid displacement in the open capillary was measured by an eyepiece micrometer through the dissecting microscope, with a resolution of $\pm$ 1 μm. Bubble movement for a 0.2-μl displacement in an open system, i.e. with no retinal "diaphragm," was 50 μm. Four measurements were made for each retinal segment.

Results

For each retinal segment measured, fluid withdrawal volumes to the point at which retinal segment deformation ceased were quite consistent. The mean volume of fluid withdrawn at which retinal deformation ceased was significantly less for central retina than for peripheral retina (center = 0.21 μl $\pm$ 0.03; periphery = 0.27 μl $\pm$ 0.02 [mean $\pm$ S.E.]; $t = 2.44$; $df = 10$; $P < 0.025$). Thus, the peripheral retina permitted a greater shape deformation before detachment than did central retina.

More fluid displacement, as indicated by bubble movement (μm) within the open capillary tube, was measured for peripheral retina for the standard pressure change (center = 28.5 μm $\pm$ 4.6; periphery = 37.9 μm $\pm$ 3.9; $t = 2.7$; $df = 10$; $P < 0.025$) (Fig. 1B). Interestingly, elasticity measures from the central and peripheral segments of the same retina were correlated ($r = 0.69$) and were related to retinal size, this correlation principally due to the smaller retinas deforming more.

Discussion

Both measures of retinal elasticity were greater for peripheral retinal segments: the peripheral retina permitted a greater maximal deformation, and for a given pressure change, showed greater deformation than the central retina. This is consistent with greater elasticity of the peripheral retina, a necessary condition for hypotheses that propose that differential stretch of the growing retina produces the area centralis.

An alternative interpretation of these results is that the decreasing radius of curvature from central to peripheral retina at this age (Mastronarde et al., 1984) is the physical parameter producing the difference in apparent deformability: since the peripheral retina curves more, it might accommodate more fluid. Although the retinal mounting technique used here appeared to make the retinas taut, this possibility cannot be discounted. This is an unavoidable problem of this measurement technique, and alternative measures subject to different problems such as whole-eye expansion (van Alphen, 1986) or linear stretch (Wu et al., 1987) could be used to constrain further interpretation of this result. The measurement technique used here has several intrinsic advantages and could be profitably combined with them: it isolates a particular tissue, unlike whole-eye expansion; and it delivers a type of expansion, convex with receptors outward, that is similar to natural growth, unlike linear applied stretch.

The sources of differential elasticity could be multiple. At this point in development, these sources could include: greater thickness of central retina compared to peripheral retina; greater overall vascularization of the central retina; more elaborate dendritic connectivity (Rapaport & Stone, 1984); and greater numbers of axons in central retina. There is direct evidence that vascularization contributes to the viscoelastic properties of the retina: in the mature bovine retina, the tangent modulus of elasticity of retinal strips containing a meridional vessel is substantially different than unvascularized strips (Wu et al., 1987). Neural cell adhesion molecules have also been reported to be distributed preferentially to the central retina in early development (Rutishauser, 1986).

The principal difficulty relating whole-eye growth to differential retinal growth is that it seems unlikely that the elastic properties of a fragile tissue like the retina (compared to other tissue components like sclera or musculature) could influence eye size and conformation. However, a recent study of the consequences of vitreal inflation of the whole eye (adult and neonatal human), with sclera dissected away and choroid exposed, had the surprising result that ciliary musculature was much more elastic than the apparently fragile choroid and retina: the choroid (and retina) retained its spherical shape and dimensions, while the region of the eye equator and the ciliary musculature stretched markedly (van Alphen, 1986). Early differences in the elasticity of the choroid and retina could therefore be possible candidates for limiting eye growth and shape, much like the bands of a radial tire constrain the shape of the tire under pressure. Alternatively, even if the retina does not provide the limit of elasticity within the layered membranes of the retina, choroid and sclera, differential elasticity of the retina during expansion might permit the retina to reconform itself within the bounds of the limiting sclera. For example, a weakened area on the inner tube of a tube tire would grow relatively larger as the whole tire was inflated, although the inner tube would retain its tubular shape.

Retinal growth could therefore be conceived of as the passive expansion of a membrane with areas of greater or lesser elasticity, which eventually produces the adult differentiated topography. It is interesting to speculate that the conformational change of the cat retina described elegantly by Mastronarde et al. (1984), in which the retina occupies a continually decreasing percentage of the sphere of the eye during development, might be due to the differential elasticity of retinal components. The experiments of van Alphen (1986) showing the ciliary musculature to be more elastic than the developing choroid and retina are consistent with this hypothesis. The consequences of integration of variable retinal elasticity with the elasticity of other components of the developing eye, including sclera, choroid, and ciliary musculature, as well as developmental changes in these parameters, are profitable avenues for future investigation.

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