The Relevance of Studies in Chicks for Understanding Myopia in Humans

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Introduction

Research on the etiology of myopia can be divided into the periods before and after animal research into myopia became prominent. During the earlier period, the predominant opinions were that myopia was either entirely of genetic origin (although there was no strong genetic evidence), or that it was entirely due to excess accommodation (with no plausible evidence linking accommodation to myopia). In addition, a few eccentrics held that myopia was a homeostatic response to a habitual near-viewing distance.

The accidental discovery during the 1970's that obscuring the view of the eye of a monkey, chick, tree shrew, or child made the eye myopic demanded an explanation of how visual experience altered the eye or brain.1–4 Subsequently, this was shown to be true for mice as well.5,6 The most important discovery that followed, showing that eyes would alter their refractive state, compensating for either negative or positive spectacle lenses, in chickens, fish, tree shrews, marmosets, rhesus monkeys, and guinea pigs,7–12 made it inescapable that there existed a homeostatic mechanism that regulated refractive error. This homeostatic mechanism posed the difficult problem that it required that the eye or brain be able to distinguish hyperopic defocus (image behind the photoreceptors) from myopic defocus (image in front of the photoreceptors). Here, human
intuition fails us. We are only able to focus a microscope or binoculars by trial and error, recalling whether the image is more in focus than it was a fraction of a second earlier; it seems impossible that the eye could use this method, especially as it seems implausible that the eye or brain could recall how sharp an image was days or months before — the time required for eye-growth to cause a detectible change in refractive status. Therefore, the visual system would seem to do better automatically than we can do consciously.

These discoveries from experiments on animals have had a curiously ambivalent effect on clinical research on myopia. On the one hand, it has made the entire community very attuned to the possible consequences of blur. Thus, there have been dozens, if not hundreds, of papers evaluating the possibility that blur caused by inadequate accommodation or higher order optical aberrations or transient myopia after long periods of reading might cause myopia. Furthermore, there has been a major, meticulously conducted clinical trial using progressive addition lenses on children to reduce the magnitude of the defocus experienced. On the other hand, the most important insight of the animal research — that there was a bidirectional homeostatic control of refractive state — has been largely ignored in the clinical community, so that only occasional studies have tested the notion that the way to counteract the effects of hyperopic blur leading to myopia is not with less hyperopic blur but with myopic blur, which may lead the eye away from myopia.

It was an accident that proved fortunate for the development of modern biology that Gregor Mendel studied the particular traits of peas that he did, and thereby discovered the simplest form of inheritance. One wonders how many other monks chose to study drought-resistance or plant-height or animal-size and failed to find a tractable experimental system. Similarly, the choice of the mold Neurospora and the fly Drosophila were fortunate choices for the study of genetics and of circadian rhythms, as were the choices of the roundworm C. elegans for cell-lineage studies and of zebra fish for developmental studies. One presumes that none of these fields would be where they are today had the researchers chosen cats or chimpanzees.

In myopia research, the accidental choice of chicks and monkeys during the 1970’s, resulting from unrelated studies on effects of experience on brain development, have proven particularly fortunate. Because this volume is largely devoted to studies of myopia in humans and because we were asked to write on “The Pivotal Role of the Chick Eye,” we
will concentrate on aspects of myopia research on chicks that bear on clinical concerns.

The Search for Error Signals

The existence of a homeostatic mechanism that guides the eye towards emmetropia requires one or more error signals that reflect how far the eye is from its “goal” or set-point. Thus, to consider what the possible visual error signals guiding eye growth might be rests on whether or not the eyes can discern the sign of defocus, instead of simply the degree of defocus. If the eye can only use the degree of defocus, many possible visual signals, such as the absence of small features in the environment (high spatial frequencies) might provide that information. Indeed, the issue becomes similar to the psychophysical question of how we can assess that an image is blurred. One could imagine that one simply assesses the amount of visual stimulation, which would be higher in focused images; or perhaps one measures the activity of neurons responsive to high spatial frequencies, which would also be higher in focused images. In the perceptual case, one can dismiss both of these possibilities, because clouds, for example, which contain neither strong contrasts nor high spatial frequencies, do not appear blurred. Instead, two psychophysical theories are in contention at present. One holds that the visual system compares the activity of neurons tuned to higher and lower spatial frequencies, that is, it estimates the slope of the function of “power” versus spatial frequency. The other holds that the visual system performs a template match of the edges at different spatial frequencies.

The blur hypothesis

In the case of eye-growth, one could argue that the eye simply elongates in proportion to the amount of blur that elongation is inhibited proportionally by retinal activity (the Blur Hypothesis). This view would equate the myopia caused by wearing negative lenses with that caused by form-deprivation, and it would explain why increasing retinal activity by intense stroboscopic illumination prevents myopia induced by form-deprivation and drives eyes toward hyperopia.

According to this view, blur could provide the error-signal guidance for people or animals that mostly view distant objects, assuming that neonates
were hyperopic and accommodation imperfect, so that the amount of visual detail, and hence the magnitude of visual neural activity, would be low at first and would increase until the eye became emmetropic. This hypothesis would not explain how myopic neonates would emmetropize; instead, it would predict that they would become progressively more myopic. Furthermore, to explain the compensation for positive lenses, this hypothesis would require that neonates mostly view nearby objects, so that the positive lenses would bring most objects into focus, thereby increasing the activity of retinal neurons, and as a result, inhibiting ocular elongation. This conjecture brings on a larger problem: Why would not all young eyes become myopic, thereby maximizing their retinal activity? Perhaps if myopia caused all nearby objects to be in focus, the inhibition of ocular elongation would be so strong that it would drive the eye back in the hyperopic direction (as the cornea and lens continued to flatten), such that only emmetropic eyes would have the level of blur that keeps the refractive error stable. It is not obvious how this optimal level would be calibrated to bring nearly all eyes close to emmetropia.

**Bidirectional lens-compensation**

The strongest challenge to the Blur Hypothesis is that at least some animals compensating for defocus imposed by eyeglass lenses can apparently compensate for both myopic and hyperopic defocus. To verify this ability, one must be certain that the eyeglass lenses put on the animal do impose opposite signs of defocus. This requires that the eye respond bidirectionally to a level of defocus greater than its distance from emmetropia. That is, if the eye is 5 D hyperopic and only responds to positive lenses of 4 D or less, one cannot test whether it truly responds to myopic defocus.

In the case of chicks, their eyes clearly distinguish myopic defocus (image in front of photoreceptors) from hyperopic defocus (image behind photoreceptors). First, chicks, with or without accommodation, compensate for positive lenses even if restrained from approaching the walls of their chamber, which are placed beyond the far point of their eyes, ensuring that all images are myopically defocused.\(^{17,18}\) Second, when chicks are wearing negative lenses, a few minutes a day of wearing positive lenses negates the whole day of wearing negative lenses. It is difficult to imagine that the sharp vision experienced in those few minutes increases the retinal activity more than the sharp vision resulting from accommodation...
over the course of the day. Third, covering positive lenses with a light dif-
fuser does not decrease the compensation for the positive lenses, even
though the same diffuser worn alone would cause myopia.\textsuperscript{18} Fourth, if
eyes are enormously blurred by wearing lenses that are $+5$ D on one axis
and $-5$ D on the orthogonal axis (Jackson Crossed Cylinders), their
refractions go slightly in the hyperopic direction (the opposite of what
would be expected by the Blur Hypothesis and the opposite of what
occurs with light diffusers), and if weak positive or negative lenses are
added to the Jackson Crossed Cylinders, the eyes compensate normally
for these lenses.\textsuperscript{19}

The cases of marmosets and fish are similar to that of chicks: The refrac-
tions of marmoset eyes change reliably in the direction that compensates
for the lenses; because the eyes were emmetropic at the start of the experi-
ments, there is no issue of which side of emmetropia the lenses put
them.\textsuperscript{20,21} In the case of fish, eyes wearing lenses that impose at least 9 D of
myopic defocus compensated by 7 D within the two-week observation
period, a change almost as great, but in the opposite direction as with
negative lenses.\textsuperscript{10}

In other species, the situation is more complicated. In guinea pigs and
tree shrews, only positive lenses of less than $+4$ D are compensated. In
guinea pigs, the eyes wearing positive lenses change in the opposite direc-
tion as those wearing negative lenses in refraction and ocular elongation,
but the eyes wearing positive lenses do not become more hyperopic than
the fellow eyes, because all lens-wear causes a small myopic shift in guinea
pigs.\textsuperscript{12} However, the loss of directional compensation with the optic nerve
section\textsuperscript{22} suggests that the intact animals may distinguish hyperopic from
myopic defocus. In tree shrews, the eyes also compensate for positive
lenses, but because the animals are $+10$ D hyperopic to begin with, the
positive lenses do not impose myopic defocus.\textsuperscript{11} In monkeys, fitting eyes
with progressively increasing powers of negative or positive eyeglass lenses
causes eyes to become more myopic or hyperopic, respectively. Even very
strong positive lenses prevent the normal loss of hyperopia. Therefore, one
can say that the positive and negative lenses cause opposite responses. The
problem with this view is that the animals could look at very nearby
objects, so that even if the eyes could not discern the sign of defocus, the
occasional clear views might be enough to keep their refractions stable. To
address this concern, in a meticulous study, Norton \textit{et al.} had tree shrews
counteract wear positive lenses for 45 minutes once a day only when their viewing
distance was controlled and their accommodation monitored (wearing
negative lenses the rest of the time). Of those wearing +5 D lenses (but not those wearing −5 D lenses), some animals grew in the hyperopic direction and others in the myopic direction, suggesting that at least some animals were compensating for true myopic defocus.

**Recovery from ametropia vs. compensation for lenses**

Although the changes in eye-growth caused by lens-wear can only have a visual origin, the same is not true for recovery from the myopia or hyperopia that results following lens-removal. Because the eye is abnormally long or short, during recovery, the eye is not only compensating for the myopia or hyperopia but is also restoring its natural shape. Thus, eye-growth can be influenced by visual and shape-restoring mechanisms, which can operate in the same or opposite directions, and they may interact. For example, it requires less daily vision for chicks to recover from 10 D of myopia than for them to compensate for lenses imposing the same defocus. The relative potency of the visual and the shape-restoring mechanisms may vary among species, ages, and conditions. Thus, making eyes myopic and then correcting the vision to emmetropia with lenses or putting the animals in darkness can either keep the eyes myopic (if only vision is at work) or can permit some recovery (if the shape-restoring mechanism is stronger than the visual one) in both chicks and tree shrews.

**The complication of the emmetropization end-point**

Another complication is that, although one tends to think of emmetropization as a process that leads to emmetropia, probably guided by an estimate of the refractive error, there is evidence from both monkeys and chicks that the initial phase of emmetropization in neonates goes to a stable refractive endpoint that is idiosyncratic for each individual, followed much later by a second phase that goes to actual emmetropia. The problem that this poses for studying lens-compensation in young animals is that, if one does not know the end-point toward which an animal’s eye is growing, one cannot be certain how to interpret the effect of the eyeglass lens. For example, if an animal is +5 D hyperopic and if the set-point the eye is growing towards is +2 D, imposing +3 D or less of myopic defocus with positive lenses would have no effect, as the lenses would simply help the eye towards its end-point, whereas if the set-point had been more hyperopic or the lens-power stronger, it would have put
the eye beyond its set-point and made it grow in the hyperopic direction, as one would expect for bidirectional lens-compensation. This individual variation in set-points may account for why some tree shrews became hyperopic and others myopic in the experiment described above in which animals wore positive lenses briefly each day.

In this regard, the chick eye is most useful because it emmetropizes to within a few diopters of emmetropia within a week of hatching and compensates for lenses from −10 D to +15 D. Therefore, if one controls the viewing distance and accommodation, one can be certain that the defocus is hyperopic with negative lenses and myopic with positive lenses, and therefore that this end-point complication does not apply.

Optical aberrations as error signals

The existence of bidirectional lens-compensation raises the question of what error signal could provide the growth-guiding signal. In a perfect optical system, one could not distinguish myopic from hyperopic defocus unless one knew the object being imaged. However, real eyes are not perfect optical systems, and so the issue is what aberrations might provide the signed error signal. Among the monochromatic aberrations (that is, the ones that exist in monochromatic light), it is well known that spherical aberration (the difference in focal length between the center and periphery of the lens) can interact with defocus to give a signal that distinguishes myopic from hyperopic defocus, so that the focused image is not necessarily the clearest. This may be responsible for the finding in some humans that the refractive error depends on the spatial frequency of the stimuli viewed. It may also account for the ability of humans to learn to discriminate whether small letters are defocused in the myopic or hyperopic direction, when presented under carefully controlled conditions. Arguing against the use of these aberrations is the fact that individual eyes have different aberrations, and the visual system would seem to have to know the aberrations of the particular eye to use them to distinguish the sign of defocus.

A simpler error signal would make use of the longitudinal chromatic aberration of the eye. Because short-wavelength (blue) light is focused more strongly than long-wavelength (red) light, if a black/white edge is in focus, the blue light will be focused in front of the photoreceptors, while the red would be focused behind the photoreceptors. Thus, if the blue aspect of the image were sharper than the red aspect, it would indicate that
the eye was defocused in the hyperopic direction (image focused behind the photoreceptors), whereas if the red aspect were sharper, it would indicate myopic defocus.

The interest in chromatic aberration as an error signal for emmetropization and lens-compensation was diminished by studies some years ago showing that chicks raised in monochromatic illumination could compensate for lenses, although no comparison of wavelengths could be made under these conditions. These findings do not imply that chromatic aberration is not used, but only that other error signals can be used. In fact, because chicks can compensate for as much as 10 D of defocus imposed by eyeglass lenses,\textsuperscript{34} the existence of other error signals is implied because chromatic aberration would provide a useful error signal only in the range of 1–3 diopters.

To make a stronger test of whether chromatic aberration is used, we arranged to have chicks presented with wallpaper that simulated the chromatic contrasts that would be present at black/white edges if the eye were myopic or hyperopic. We found clear evidence that the eye grew in the direction that compensated for the simulated refractive error.\textsuperscript{35} This result shows that chromatic cues can be used to distinguish myopic from hyperopic defocus because no other cues were available from this simple striped wallpaper.

Because there is credible evidence that humans use chromatic aberration to determine in which direction to accommodate,\textsuperscript{36} it is plausible that humans use chromatic aberration in emmetropization as well. The fact that both chickens and humans have cones sensitive to blue, green, and red light argues that chicks may, in this regard, be more similar to humans than most mammals, which have only two cone-types.

**Other possible visual error signals**

The possible error signals are limited by the reader’s imagination, maybe not even by that. The aberration of astigmatism results in light being focused at two different planes, much like chromatic aberration. If the eye knew the sign of its astigmatism, it could sense changes in the sign of its defocus by a change in which lines were sharper. If the eye could compare the image quality in the ventral visual field (which is myopic\textsuperscript{37}) with that in the central visual field, it could determine whether it was myopic or hyperopic. If it could keep track of the changes in image quality with accommodation, this too could yield the sign of defocus, as could
decoding the fluctuations in retinal position caused by oscillations in blood flow or intraocular pressure. Unfortunately, none of these possible signals have been put to a test.

One signal that has been put to a test is that the ON and OFF pathways of the visual system seem differentially able to affect the compensation for positive and negative lenses in chicks.\(^38\)

**How Important is Having a Fovea?**

One of the attributes of the chicken eye that makes many view it as an inappropriate model for human myopia is the relatively uniform distribution of photoreceptors across the retina, in contrast to the steep gradient of photoreceptor density as a function of retinal eccentricity in primates. This uniformity helps make it believable that local parts of the retina can adjust their own refractive state rather independently from other regions, as shown by experiments with partial diffusers or with lenses covering part of the retina.\(^39,40\) Subsequently, it has been found that monkeys also adjust the expansion of parts of the eye locally, very much like chicks.\(^41\)

The obvious importance of the fovea may be another instance in which human intuition fails. Although our subjective awareness of the visual world is dominated by what we see with the fovea, the foveal area is so small that it contains few neurons. For example, although the parasol retinal ganglion cells are 100 times more concentrated in the foveal region than in the periphery, their total number increases with distance from the fovea, simply because the retinal area increases. Indeed, there is no clear evidence that the fovea has a privileged role in control of eye growth in humans. It has been known since 1931 that individuals differ considerably in the refractions of the peripheral retina\(^42\); furthermore, a longitudinal study in 1971 showed that those adolescents with hyperopic refractions in their peripheral retina were much more likely to become myopic than those with myopic refractions in their peripheral retina.\(^43\) It is an open question whether peripheral hyperopia causes myopia, and, if it does, whether it is because the hyperopia results from the eye compensating for myopic defocus in the periphery or because the eye has an elongated shape caused by myopia in the central retina.\(^44\)

One cannot overstate the importance of the finding, first in chickens and then in primates, that the peripheral retina plays an important role in experimental myopia. The implication of this role for human myopia is
threefold: First, it means that it may be futile to look for the etiology of myopia in the small degrees of defocus that occur at the fovea, for example during reading, when much larger degrees of defocus are present in the periphery. Second, it forces one to accept that any particular point on the retina will experience an alternation of myopic and hyperopic defocus depending on whether the fovea (which largely controls accommodation) is looking at an object closer or further than that particular peripheral point. Third, it means that, as Ian Flitcroft has argued, when one is outdoors the visual world is relatively flat in dioptric terms, in that nearly everything is more than a meter away. Thus, no point on the retina would be more than 1 diopter defocused relative to the point viewed with the fovea. In contrast, the range of focal planes indoors is much greater so that when viewing a nearby object like a book, the visual scene surrounding the book can be several dioptrers defocused in the myopic direction, or, if one focuses in the distance, the book can be several diopters defocused in the hyperopic direction. This difference is likely to be more important than the difference in light intensity between indoor and outdoor vision as a factor in the etiology of myopia.

The awkward aspect of accepting the likely importance of peripheral defocus in human myopia is that one cannot easily measure or control the temporal pattern of defocus that would be experienced by each retinal locus. In chickens, it is possible to begin to assess the effect of alternation of myopic and hyperopic defocus by alternating strong positive and negative lenses. We found that, even though positive and negative lenses have approximately equal effects when worn alone, the positive lenses have a much greater effect than the negative lenses, when the lenses are alternated. Indeed, even a few minutes of positive lens-wear four times a day can balance out the remainder of the day wearing negative lenses. Furthermore, this asymmetry in the efficacy of the positive and negative lenses depends on the frequency of alternation: if myopic and hyperopic defocus is alternated several times a second, the asymmetry disappears.

To get to the mechanism underlying these alternation effects, we studied chicks with different periods of lens-wear alternating with different periods of darkness. We found that periods of lens-wear less than two minutes were without effect. Because chicks accommodate for only brief periods, this may explain why accommodation seems to have little effect on emmetropization or lens-compensation. Furthermore, we found that there was a great difference in how long the intervals between lens-wearing bouts could be before lens-compensation was lost: positive lenses...
inhibited ocular elongation for much longer periods than negative lenses stimulated it. This probably explains the stronger effects of positive lenses when they are alternated with negative lenses, but it does not explain the similar effects of brief periods of positive and negative lenses when worn alone. Although there are some temporal similarities among the responses of chicks, tree shrews and monkeys, it is likely that other relevant temporal parameters may differ among species.

The implication of these complex timing effects is that one might be on the wrong track by looking at the amount of time that children spend reading as a risk-factor for myopia; the more important aspect may be the particular temporal pattern with which a child alternates reading and looking up from reading. Indeed, reading may itself involve an alternation of myopic and hyperopic defocus both in the fovea and the periphery because when a child looks up from reading he or she may experience a transient near-work induced myopia. Although of course we cannot impose different temporal patterns of alternation of myopic and hyperopic defocus in children, we may be able to obtain clinically useful information by studying the temporal pattern of reading shown by children that become myopic compared with those who do not.

**Mechanisms of Emmetropization**

The two most prominent changes in the eye that contribute to emmetropization and its laboratory analogue, eyeglass-lens compensation, are changes in ocular length (with associated scleral remodeling) and changes in choroidal thickness. If the eye elongates more than usual or if the choroid thins, the retina is pulled backward, making the eye more myopic. Conversely, if the eye slows its elongation while the cornea and lens continue to grow (thereby increasing their focal length), the eye will become less myopic, as would occur if the choroid thickens. Wearing positive eyeglass lenses, which put the image in front of the photoreceptors, causes the choroid to thicken and the ocular elongation to slow, both acting to bring the image back onto the photoreceptors, as do the opposite changes if negative lenses are worn.

To understand how homeostatic control of eye growth brings the normal eye towards emmetropia and how disturbances of this process cause myopia, an understanding of the signaling between the tissues of the eye is important. Although visual control of eye growth can occur within the
eye, without the influence of the brain,\textsuperscript{52,53} it appears that, at least in guinea pigs, the detection of the sign of defocus requires the brain to be connected to the eyes.\textsuperscript{22} Whether or not the control of eye-growth is local or brain-mediated, the retina must signal the defocus, and the choroid must conduct or create the signals reaching the sclera. We will now discuss each of these tissues.

**Scleral similarities and differences between humans and chickens**

The sclera of chickens differs from that of humans. The chicken has the classic vertebrate sclera, consisting of a layer of cartilage surrounded by layers of fibrous connective tissue, whereas in most mammals, including primates and rodents, the cartilage has been lost, although molecular traces of its existence persist.\textsuperscript{54} One can speculate that the loss of the cartilage in early mammalian evolution was innocuous because the precursor of Eutherian mammals had small eyes, which did not require the reinforcement of the cartilage. However, the consequence of the loss of cartilage is that whereas birds can have large eyes despite thin (about 120 µm) scleras, mammals with large eyes, such as elephants, have grotesquely hypertrophied scleras, as much as 8 mm thick,\textsuperscript{55} apparently required to maintain the shape of the large eye (reviewed by McBrien and Gentle).\textsuperscript{56}

Despite this difference in scleral anatomy, the fibrous sclera of mammals and the fibrous layer of the avian sclera appear to grow similarly. When ocular elongation accelerates, the fibrous sclera thins and loses material both in mammals\textsuperscript{57,58} and birds.\textsuperscript{59,60} The cartilaginous layer of the sclera of birds, however, increases its thickness as the eye elongates, and this is accompanied by an increase in synthesis of proteoglycans.\textsuperscript{61–63,60} Because the cartilaginous layer dominates biochemical measurements of scleral growth, it can mislead one to conclude that the avian sclera grows oppositely to the mammalian one.

Although one might expect that the cartilage-reinforced sclera of birds would affect the shape the eye could take, when chicks\textsuperscript{64} and monkeys\textsuperscript{65} have half of their visual field covered by a diffuser, the eyes expand only in the visually deprived half, with the boundary between the visually experienced and visually deprived halves being approximately equally sharp in both species. This is further evidence that the tissue differences between
the two types of sclera do not imply completely different mechanisms of ocular growth regulation.

What then is the relation between the two layers of the sclera? If the fibrous and cartilaginous layers from an eye growing toward myopia (or towards hyperopia) are dissected apart, and each layer is co-cultured with the opposite layer from a normal eye, it is the fibrous layer that determines the rate of growth of the “recipient” cartilaginous layer; the condition of the “donor” cartilaginous layer does not affect the growth of the “recipient” fibrous layer. If it is the general condition that the fibrous layer controls the cartilaginous layer, one could suppose that when, in the course of mammalian evolution, the cartilaginous layer was lost, eye growth regulation would not have required a major change in the growth control of the sclera.

These results should not be taken to imply that the fibrous sclera completely controls the growth of the cartilaginous sclera. We have recently found that the fibroblast growth factor causes the fibrous layer to increase its synthesis of proteoglycans and causes the cartilaginous layer to do the opposite, when the different layers are cultured separately. It is unclear where this growth factor comes from under natural conditions because it is also found in the retina, in the nerve fiber layer and inner plexiform layer, and in choroidal microvascular endothelium and RPE cells.

Retinal signals

In part because of the ease of doing experiments on chicks, there is more known about possible retinal signals that might be registering the degree and sign of defocus in chicks than in other species. Of particular interest are molecular signals that go in opposite directions when negative vs. positive lenses are worn.

Glucagon-insulin

The first of these potential signals discovered was glucagon, in that the transcription of the immediate early gene ZENK (also known as Egr1, among other names) increases in glucagonergic amacrine cells, when positive lenses are worn and decreases when negative lenses are worn, these changes being independent of both illuminance levels and chromatic cues. Furthermore, exogenous glucagon blocks both the excessive ocular
elongation and choroidal thinning caused by negative lenses\textsuperscript{73} and form deprivation.\textsuperscript{74}

Curiously, in the eye, as in the liver, insulin has effects opposite to those of glucagon: it counters the effect of positive lenses, and even in normal eyes, increases ocular elongation and thins the choroid.\textsuperscript{75,73} Furthermore, glucagon at concentrations too low to have an effect by itself can attenuate the effects of insulin, and \textit{vice versa}.\textsuperscript{73} Injections of glucagon suppress the proliferation of retinal progenitors in peripheral retina, while injections of insulin do the opposite.\textsuperscript{76} These findings support the hypothesis that glucagon and insulin may be output signals from the retina that decode the sign of defocus and modulate eye growth. The site of action of both may be on retinal pigment epithelial cells, where glucagon and insulin receptors have been found, or on the sclera, which has insulin receptors (glucagon:\textsuperscript{77,78}; insulin:\textsuperscript{79,80}).

Interestingly, there is a subclass of glucagonergic amacrine cells in young chicks that are located in the peripheral retina, which send axons to the far peripheral retina. Because these cells are concentrated near the equator of the eye, and because injections of glucagon suppress equatorial eye growth, it may be that the expression of glucagon in these neurons determines the equatorial expansion of the eye,\textsuperscript{78} whereas neurons in the posterior retina may control axial elongation.

At present it is unclear whether there is a primate version of this signaling mechanism. Glucagon has not been found in primate retinal neurons, although the expression of the transcription factors erg-1 and fra-2 was found to be decreased in ON-bipolar cells and a subclass of GABA-ergic cells in primate retinas of eyes wearing diffusers, whereas erg-1 (but not fra-2) increased in eyes wearing +3 D lenses, which corrected the eye’s normal hyperopic refractive error,\textsuperscript{81} suggesting that in-focus images stimulate expression of these transcription factors more than blurred or diffused images. However, because visual stimulation changes the expression of many transcription factors in many retinal neurons, this difference may reflect the amount of retinal stimulation rather than a specific signal related to defocus.

\textbf{Retinoic acid}

Retinoic acid is a metabolic product of vitamin A with a myriad of critical roles during development. In the retina, retinoic acid increases if eyes are made to accelerate their elongation by wearing diffusers or negative lenses,
and decreases if eyes are made to slow their elongation (chicks:82,85, guinea pigs83). Inhibiting synthesis of retinoic acid reduces form-deprivation myopia.84 As will be discussed below, synthesis of retinoic acid by the choroid also depends on the direction of eye-growth, but the synthesis in the retina may be uncoupled from that in the choroid, perhaps because the retina does not secrete much retinoic acid.85 Therefore, retinal retinoic acid may be more of an indication of other retinal functions than of a signal acting on other ocular tissues.

**Dopamine**

Dopamine is a neurotransmitter used by specific amacrine cells in both chick and monkey retina. The levels of dopamine are reduced in eyes wearing diffusers86 and negative lenses,87 and increased in eyes recovering from form-deprivation myopia.88 Injections of apomorphine, a non-specific dopamine agonist, inhibit the development of form-deprivation myopia86,89,90 and lens-induced myopia91 in both chicks and monkeys, suggesting a similar role for both species. The mechanism is presumably mediated via the D2 receptors, as a D2-specific agonist, but not a D1 agonist, is effective in inhibiting deprivation-induced myopia.92

Despite the promising nature of these results, the findings that reducing dopamine action either by haloperidol, an antagonist, or 6-hydroxydopamine, which depletes dopamine, also suppresses myopia, casts doubt on dopamine being a primary growth-inhibitory signal molecule.92 It remains possible, however, that the conflicting evidence obtained from these other studies might be a reflection of actions on dopamine receptors in different tissues, such as the RPE or choroid.

**Acetylcholine**

A third potential retinal signal molecule is acetylcholine, muscarinic antagonists of which have been used to prevent myopia in humans for many years.93–95 Although originally thought to act on the ciliary muscle preventing accommodation, it is now clear that this is not the case because atropine and pirenzepine also inhibit the development of lens- and diffuser-induced myopia in chickens,96–99,91 in which accommodation is not mediated by muscarinic receptors. Although the site of action of these drugs is unknown, evidence for its being retinal is weak: neurotoxin depletion of the cholinergic amacrine cells does not alter the eye’s response to
form deprivation, nor does it alter the effects of atropine. Muscarinic receptors are found in every tissue of the eye.

**Choroidal signals**

Although it is clear that any molecule influencing scleral growth must originate in or pass through the choroid, the phenomenon that brought attention to the physiological state of the choroid was the dramatic thickening that occurred when chick eyes were exposed to myopic defocus either imposed by positive lenses or prior form-deprivation. This thickening could cause the choroid to increase in thickness by as much as a millimeter, four times the normal thickness, accounting for at least half of the refractive compensation to the lens. This response was also found in rhesus monkeys, marmosets and guinea pigs, but to a much smaller extent, having little refractive effect.

Although one is tempted to dismiss the choroidal changes in mammals as insignificant, because they are so small, there is evidence from chicks that the state of the choroid has a profound influence on the state of the sclera, and hence on the growth of the eye. Specifically, if one takes the choroid from an eye with imposed myopia or hyperopia and cultures it with sclera from an untreated eye, the sclera responds in the direction predicted by the choroid from which it came: The rate of synthesis of DNA and proteoglycans in the sclera is increased in scleras incubated with choroids from eyes becoming myopic and decreased in scleras with choroids from eyes becoming hyperopic. By the same token, fluid aspirated from the choroid of slower-growing eyes recovering from deprivation myopia inhibited scleral proteoglycan synthesis while fluid from eyes becoming myopic stimulated synthesis.

This choroidal modulation of scleral growth is likely the result of choroidal secretion of signals to which the sclera is sensitive. Potential molecules include retinoic acid, transforming growth factor-beta (TGF-beta), and ovotransferrin, all of which have their choroidal content changed by whether the eye is growing towards myopia or hyperopia, as will be discussed. In addition, the choroid secretes tissue plasminogen activator, tPA, which stimulates the production of metalloproteinases and collagenases, which degrade extracellular matrix components, as would be involved in the remodeling of the sclera.

Retinoic acid furnishes a particularly clear example of how this choroidal modulation of scleral growth might work. The chick choroid,
The overall similarity of the effects of visual experience on eye-growth in birds and mammals suggests that the underlying mechanisms are probably conserved. How, then, is one to reconcile the order-of-magnitude
differences in the defocus-induced choroidal thickness changes between birds and mammals with the presumed similarity of control of eye growth? One possibility is that the choroid contains two independent tissues: the stroma, which thickens and thins in response to changes in refractive state$^{101}$ as a result of changes in the volume of the lymphatic lacunae,$^{114}$ and a secretory tissue, perhaps the lamina fusca, which faces the sclera. If this is so, the choroidal thickening response would be independent of the secretory functions of the choroid. Alternatively, the choroidal thickening and thinning may reflect the physiological state of the choroid, which might determine what growth-modulating molecules are secreted. The evidence in favor of this coupling is that a variety of visual manipulations that inhibit ocular elongation also cause a transient choroidal thickening lasting only a few hours, which would generally not be detected in eye-growth experiments lasting days. For example, wearing negative lenses causes chick eyes to rapidly elongate and become myopic, but removal of the lenses for two hours per day cancels both of these effects and causes a transient choroidal thickening.$^{115}$ Inhibiting this daily thickening by preventing nitric oxide synthesis causes the eye to continue to elongate as though wearing the negative lenses continuously.$^{116}$ It would be interesting to see if the same transient changes in choroidal thickness are associated with inhibition of elongation in primates.

Diurnal rhythms and control of ocular growth

During growth of a tissue, cells generally alternate between dividing and synthesizing. In the case of tissues like the sclera, this means that cells stop synthesizing extracellular matrix when they are dividing. If individual cells divided asynchronously, this alternation would not be evident in any measure of growth, either at the tissue synthetic level or at the organ level. However, it is clear not only that the eye elongates with a daily rhythm, growing more during the day than at night,$^{117-119}$ but also that the growth of isolated pieces of sclera is controlled by a circadian oscillator.$^{120}$ This means that there must be substantial synchrony of the chondrocytes within the tissue. There are two lines of evidence that this diurnal growth rhythm is modulated by vision.

First, dopamine and melatonin constitute a reciprocal system that mediates ocular diurnal rhythms, with dopamine being the “day” signal and melatonin the “night” signal, controlling rod and cone sensitivity, retinomotor movements, and pigment dispersal in RPE cells (review:$^{121}$).
Both molecules seem to be involved in the visual control of eye-growth: day-time, but not night-time, levels of dopamine are reduced by form-deprivation, and apomorphine, a non-specific dopamine agonist, inhibits the development of myopia induced by wearing diffusers or negative lenses in both chicks and monkeys. Melatonin is a potent modulator of retinal dopamine release, but also has receptors in the cornea, lens, choroid, and sclera. Systemic administration of melatonin at night resulted in a significant increase in vitreous chamber depth in normal chick eyes and choroidal thinning in form-deprived eyes, and one of the three types of melatonin receptors is increased in the retina/RPE/choroid in form-deprived eyes.

Second, in growing chick eyes, there are diurnal rhythms in choroidal thickness and in the rate of ocular elongation, the phases of which are nearly opposite, with the choroid being thickest at night and the eye longest during the day. Inhibition of ocular elongation by positive lenses shifts the two rhythms into near-synchrony, whereas acceleration of ocular elongation shifts the rhythms into anti-phase. The phase difference between the rhythms in axial length and choroid thickness predicts the rate of growth on the following day in individual animals when the sign of defocus is switched from myopic to hyperopic or vice versa.

Although equivalent studies have not been done on mammals, there are rhythms in axial length and choroidal thickness in humans and marmosets. When marmosets are young, with rapidly elongating eyes, the two rhythms are in approximate anti-phase, whereas in older adolescents, in whom growth has slowed, the rhythms are closer in phase, analogous to the patterns seen in chicks with different rates of ocular growth. If choroidal thickness is correlated with the molecules that the choroid is releasing, as suggested in the previous section, perhaps these molecules stimulate ocular elongation more at one portion of the cycle than at another, or perhaps modulators of metalloproteinases involved in scleral remodeling, such as tPA, may be more effective at certain points in the daily cycle. Finally, the effect of bright light outdoors in preventing chicken and human myopia may act by stimulation of dopamine release.

Conclusions

The eyes of a wide variety of vertebrates adjust their growth using visual cues. The pervasive similarities in the mechanisms shown to operate in
chicks and primates suggest that the emmetropization machinery has been highly conserved in evolution. The bidirectional modulation of eye growth by hyperopic and myopic defocus in disparate species suggests that the same may occur in children. This possibility should not be ignored in considering what might make children myopic and what could be done to prevent it. Furthermore, the similar local effects in chicks and monkeys of defocus limited to one region of the retina implies that one must study the peripheral refractions in humans to understand the etiology of myopia. This, in turn, implies that one must consider the effects of alternating periods of myopic and hyperopic defocus, because all regions of the retina are continuously exposed to these alternations with the exception of the fovea, which is kept more-or-less in focus by ocular accommodation. From the animal work, it appears likely that understanding the spatial and temporal distribution of defocus will go a long way to understanding human myopia.

References


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