

# Retinal dopamine and form-deprivation myopia

(myopia/retina/chick)

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**ABSTRACT** Investigation of retinal neurochemistry in a well-defined chick model of form-deprivation myopia indicated that dopamine and its metabolite 3,4-dihydroxyphenylacetic acid are reduced in myopic as compared to control eyes. The reduction in retinal dopamine is evident only during light adaptation and is accompanied by a decreased rate of dopamine biosynthesis. To test whether the alteration in dopamine metabolism is related to eye growth, agents known to interact with dopamine receptors were administered locally to deprived eyes. Remarkably, the expected growth in the axial dimension was reduced, while that in the equatorial dimension was not. Therefore retinal dopamine may participate in the pathway linking visual experience and the postnatal regulation of the eye's growth in the axial dimension. The mechanism for control of chick eye growth in the equatorial dimension remains unknown.

Eye growth during normal childhood development coordinates with progressive changes in the optical power of the cornea and lens to maintain image focus on the plane of the retina (1). Observations after unilateral visual deprivation have indicated that retinal image quality influences postnatal growth. Deprivation of form vision in juvenile monkeys (2-4), chicks (5-9), or humans (10-12) disrupts normal regulation and leads to excessive eye size; distant images now focus in front of the retina, causing a myopic refractive error. This link of visual quality to eye size implicates the nervous system in growth control. Moreover, recent observations hint that such control is largely local. (i) Form-deprivation myopia in both monkeys and chicks takes place even after optic nerve transection interrupts the direct pathway from retina to brain (3, 13). (ii) Application of a partial occluder in chicks to restrict vision either in the nasal or temporal visual field induces excessive eye growth only along the corresponding ocular dimension. For example, occlusion of the nasal visual field causes excessive growth of the temporal part of the globe (14-16). We now report in avian myopia that neonatal deprivation of form vision alters retinal dopamine metabolism at the same time as the eye enlarges. Under the identical condition, ocular administration of dopamine-related agents hinders the expected elongation of the eye in the axial but not in the equatorial dimension. These findings buttress the hypothesis of local growth control and suggest the participation of retinal dopamine in the regulatory sequence. They also speak for separate mechanisms underlying the regulation of axial and equatorial growth of the eye.

## MATERIALS AND METHODS

We induced form-deprivation myopia in day-old White Leghorn chicks under aseptic conditions and ether anesthesia using one of three unioocular procedures: eyelid suture (6, 8),

translucent plastic goggle, or transparent but image-degrading plastic goggle (7). Maintained on a 12-hr light/dark cycle, the birds were killed at ages up to 4 weeks by decapitation or by perfusion with Zamboni's fixative (17) under deep pentobarbital anesthesia. Axial and equatorial dimensions of unfixed eyes were measured with vernier calipers. Thirty minutes before death, some birds received *m*-hydroxybenzylhydrazine (Sigma).

For biochemistry, retinas were sonicated in cold 0.1 M HClO<sub>4</sub> and analyzed by high-performance liquid chromatography with electrochemical detection (18). For histochemistry, retinas were processed either by the formaldehyde-induced-fluorescence technique for catecholamines (19) or by indirect immunohistochemistry for serotonin (20).

For drug therapies, one eyelid of newborn chicks was sutured; and apomorphine hydrochloride (Sigma), haloperidol (McNeil Pharmaceutical, Spring House, PA), or saline was administered daily to the deprived eye. In all instances, the contralateral control eye received saline vehicle. All agents were given under ether anesthesia by subconjunctival injection, a highly effective method of obtaining ocular drug penetration.

## RESULTS AND DISCUSSION

As previously reported, unilateral visual deprivation by lid suture, translucent goggle, or transparent goggle resulted in excessive eye growth in both axial and equatorial dimensions (Fig. 1) (5-9). All three types of visual deprivation also reduced retinal concentrations of the catecholamine dopamine and its metabolite 3,4-dihydroxyphenylacetic acid (DOPAC), as measured in light-adapted birds at intervals during a 4-week observation period (Fig. 2). Retinal concentrations of dopamine and DOPAC normally vary in accordance with the state of light/dark adaptation (21). Visual deprivation by translucent goggles for 2 weeks lessened the usual light-associated rise (Fig. 3). In contrast, no orderly change in retinal concentration of the indoleamine serotonin and its metabolite 5-hydroxyindoleacetic acid was observed in the same birds (data not shown).

Histochemical observations paralleled the biochemical results (data not shown). Control and deprived contralateral eyes were examined by the formaldehyde-induced-fluorescence technique for catecholamines. The overall fluorescence intensity of the retina tended to be greater in control eyes compared to contralateral eyes visually deprived by lid suture at either 2 or 4 weeks. In these preparations, there was no evident difference in the distribution of fluorescent dopaminergic amacrine cells or their processes. In other experiments, no difference was found in immunohistochemical reactivity of the retina for serotonin in comparing control to deprived eyes.

Abbreviation: DOPAC, 3,4-dihydroxyphenylacetic acid.

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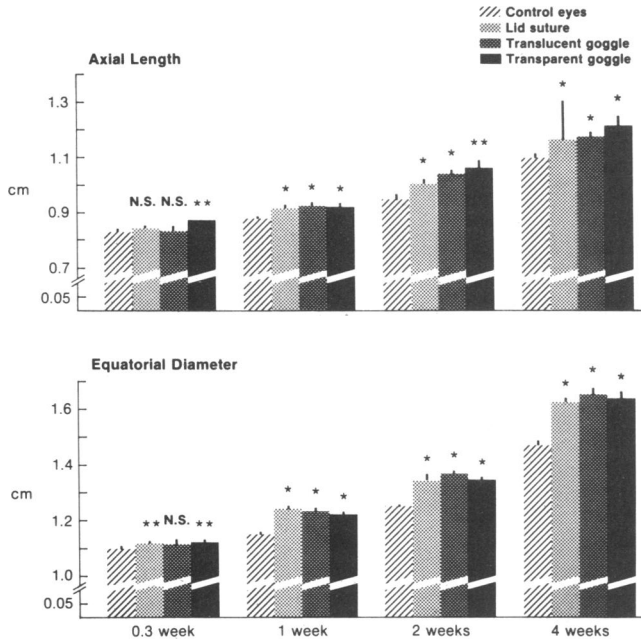


FIG. 1. Effect of visual deprivation on ocular growth. Newborn White Leghorn chicks underwent unilateral visual deprivation by lid suture, translucent goggle, or transparent goggle. Unilateral visual deprivation results in excessive eye growth in both axial and equatorial dimensions (mean  $\pm$  SEM;  $n = 5-13$  birds in each group). Student's *t* statistics were used to compare paired differences between deprived versus nondeprived eyes. N.S., not significant. \*,  $P \leq 0.001$ ; \*\*,  $P \leq 0.01$ .

To elucidate the metabolic alteration underlying our observation, we measured the retinal activity of tyrosine hydroxylase, the rate-limiting enzyme in the biosynthesis of dopamine, in light-adapted birds visually deprived for 2 weeks by unilateral translucent goggle. We did so by blocking

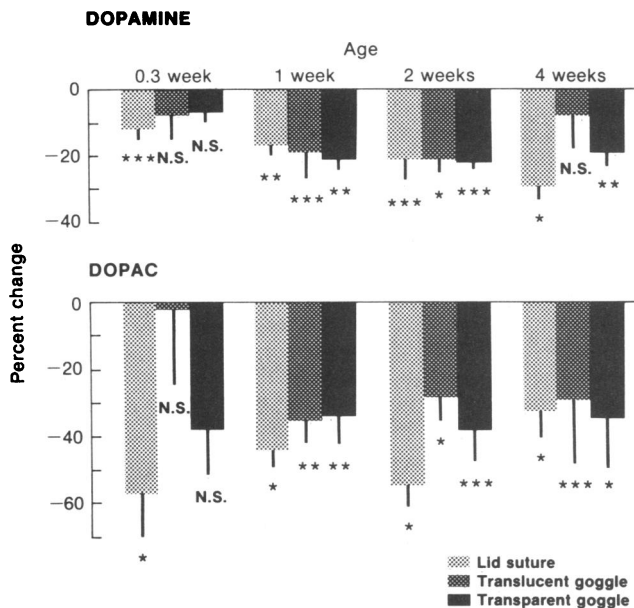


FIG. 2. Effect of visual deprivation on retinal dopamine and DOPAC concentrations. Retinal concentrations of dopamine and its metabolite DOPAC were measured in light-adapted birds after unilateral visual deprivation by one of three methods (mean  $\pm$  SEM;  $n = 7-18$  birds in each group). Deprived eyes are compared with the contralateral nondeprived eyes by means of Student's *t* statistics on the paired differences. N.S., not significant. \*,  $P \leq 0.001$ ; \*\*,  $P \leq 0.01$ ; \*\*\*,  $P \leq 0.05$ .

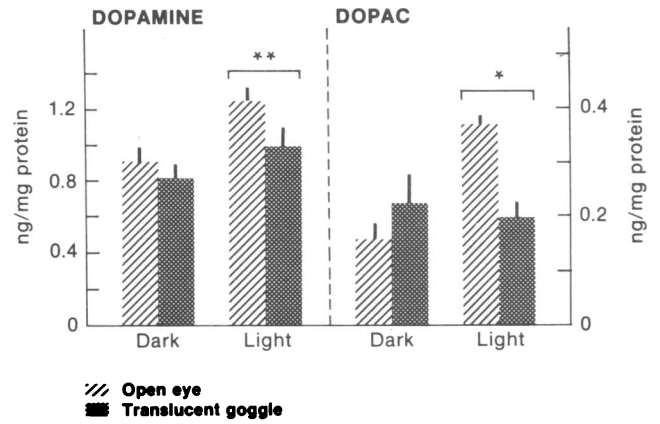


FIG. 3. Effect of visual deprivation on the light-induced rise in retinal dopamine and DOPAC. Fourteen newborn chicks, maintained in a 12-hr light/dark cycle, underwent unilateral visual deprivation by translucent goggle for 2 weeks. Seven birds were sacrificed during the last hour of the 12-hr light period; the other seven were sacrificed after 2 hr into the dark period. In nondeprived eyes, both dopamine and DOPAC levels are higher in the light-adapted retinas. Visual deprivation inhibits the rise in both dopamine and DOPAC associated with light adaptation (mean  $\pm$  SEM). Student's *t* statistics were used to compare the paired differences. \*,  $P \leq 0.001$ ; \*\*,  $P \leq 0.05$ .

the conversion of dopa to dopamine with administration of *m*-hydroxybenzylhydrazine (150 mg/kg i.p.), an inhibitor of aromatic amino acid decarboxylase (22). Thirty minutes later, the dopa concentration in visually deprived retinas ( $0.22 \pm 0.01$  ng/mg of protein; mean  $\pm$  SEM) was half that measured in contralateral eyes ( $0.43 \pm 0.03$  ng/mg of protein;  $P \leq 0.001$ , using Student's *t* statistics on the paired differences;  $n = 9$  birds), indicating a decreased rate of dopamine synthesis.

In an attempt to understand the biological implications of our observations, we administered either apomorphine or haloperidol, a dopamine agonist and antagonist, respectively. Although both are considered relatively nonselective, each shows somewhat greater affinity for the  $D_2$  compared with the  $D_1$  dopamine receptor subtype (23). Apomorphine lessened the expected axial elongation of the lid-sutured eye in a dose-dependent fashion (Table 1). At the highest dose (250 ng), apomorphine blocked lid-suture-induced axial elongation completely. Moreover, its effect was nullified by coadministration of the dopamine receptor antagonist, haloperidol, suggesting the involvement of dopamine receptors. Haloperidol alone produced a partial decrease in axial elongation, statistically significant when the treatment groups were combined; however, its effect was neither dose dependent nor significant ( $P > 0.05$ ) at any of the individual doses tested. We did not specifically evaluate whether these drugs influenced solely the axial dimension of the vitreous chamber or whether they also affected the much smaller anterior chamber. Most remarkably, all of the pharmacological treatments were selective; none influenced the exaggerated equatorial growth that takes place behind a lid suture.

Thus, deprivation of form vision in the newborn chick simultaneously perturbs ocular growth and retinal dopamine metabolism. Reduced retinal dopamine in deprived eyes is observable only during light adaptation and is associated with a decrease in tyrosine hydroxylation. Administration of apomorphine or haloperidol to an eye can reduce and sometimes even rectify the exaggerated axial growth that accompanies visual deprivation by lid suture. In contrast, neither agent corrects the exaggerated equatorial growth that occurs simultaneously. This pronounced geometric selectivity clearly points toward differential regulation of axial and

Table 1. Effect of drug therapy on the growth of lid-sutured eyes

Drug	Dose, ng	Ocular dimensions (deprived eye minus control eye)*		n
		Axial length, mm	Equatorial diameter, mm	
Apomorphine	250	-0.01 ± 0.06	0.94 ± 0.08	15
Apomorphine	25	0.09 ± 0.09	0.99 ± 0.06	11
Apomorphine	2.5	0.17 ± 0.17	0.81 ± 0.08	7
Haloperidol	300	0.18 ± 0.06	0.98 ± 0.07	15
Haloperidol	30	0.14 ± 0.09	0.99 ± 0.06	10
Haloperidol	3	0.17 ± 0.12	0.94 ± 0.08	6
Apomorphine plus haloperidol	25 30	0.51 ± 0.09	0.91 ± 0.09	8
Saline		0.36 ± 0.05	0.86 ± 0.08	13

Based on a one-way analysis of variance, there is a significant treatment effect on axial length ( $P < 0.0002$  for the apomorphine treatment groups vs. control;  $P < 0.002$  for the haloperidol treatment groups vs. control) and no significant difference between the apomorphine and haloperidol groups. In contrast, there is no significant treatment effect on equatorial diameter. The proportion of variability in axial length due to treatment is 25%; the proportion of variability in equatorial length is 4%. Tukey's "Studentized range" test at the 0.05 level identifies significant differences for the saline control vs. apomorphine (250 ng), for the combined apomorphine/haloperidol vs. apomorphine (250 ng), and for the combined apomorphine/haloperidol vs. apomorphine (25 ng) treatment groups.

\*Values are reported as mean ± SEM.

equatorial growth of the avian eye. Whether such a discriminative drug effect ultimately derives from the regional specializations in the retina of the laterally placed chick eye (24) or from a more general phenomenon applicable to eye growth in other species remains to be established.

That agents considered agonists and antagonists appear to act individually in the same selective sense to rectify axial but not equatorial growth after local administration to lid-sutured eyes presents an apparent paradox. As dopamine functions as both a neurotransmitter and a neuromodulator in the retina (25) many changes in related receptor systems and other transmitters may accompany the alterations in retinal dopamine metabolism that follow visual deprivation. Thus, it seems probable that dopamine itself is not a final mediator of ocular growth. More likely, it participates in a complex pathway linking visual experience to the postnatal regulation of axial growth of the eye.

As an alternative explanation, potential effects of dopamine or related compounds on intraocular pressure must be considered. In mammals they may well influence intraocular pressure; unfortunately, interpretation of the pressure-effect studies is hampered by their contradictory nature (for review, see ref. 26). Comparable studies are not available for the bird. In the absence of direct measurements, altered intraocular pressure seems an unlikely mediator for disparate growth patterns such as the spatially selective equatorial growth found in our study after drug therapy or the local nasal or temporal ocular enlargement that follows partial visual field deprivation (14–16).

The present report complements two recent studies on retinal neurochemistry in the primate following comparable visual deprivation. In the first, rhesus (*Macaca mulatta*) and stump-tailed (*Macaca arctoides*) monkeys with lid-fusion myopia showed an increase of vasoactive intestinal polypeptide but not of substance P in retinal amacrine cells of myopic eyes (27). In the second, monocular occlusion by an opaque contact lens in infant rhesus monkeys reduced retinal dopa-

mine, DOPAC, and tyrosine hydroxylase activity; unfortunately, neither refractive data nor eye-size measurements were included, limiting interpretation of these results (28). Certainly, these findings in the primate will stimulate investigation of retinal neuropeptides in avian myopia. Similarly, the chick and monkey results justify experiments to search for an influence of dopamine on the regulation of postnatal growth of the primate eye. Ultimately, such studies will clarify the role of the retina in determining the refractive state of the eye.

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