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Studies on the Role of the Retinal Dopamine/Melatonin System in Experimental Refractive Errors in Chickens

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We have found that development of both deprivation-induced and lens-induced refractive errors in chickens implicates changes of the diurnal growth rhythms in the eye (Fig. 1). Because the major diurnal oscillator in the eye is expressed by the retinal dopamine/melatonin system, effects of drugs were studied that change retinal dopamine and/or serotonin levels. Vehicle-injected and drug-injected eyes treated with either translucent occluders or lenses were compared to focus on visual growth mechanisms. Retinal biogenic amine levels were measured at the end of each experiment by HPLC with electrochemical detection. For reserpine (which was most extensively studied) electroretinograms were recorded to test retinal function [Fig. 3(C)] and catecholaminergic and serotonergic retinal neurons were observed by immunohistochemical labelling [Fig. 3(D)]. Deprivation myopia was readily altered by a single intravitreal injection of drugs that affected retinal dopamine or serotonin levels; reserpine which depleted both serotonin and dopamine stores blocked deprivation myopia very efficiently [Fig. 3(A)], whereas 5,7-dihydroxy-tryptamine (5,7-DHT), sulpiride, melatonin and Sch23390 could enhance deprivation myopia (Table 1, Fig. 5). In contrast to other procedures that were previously employed to block deprivation myopia (6-OHDA injections or continuous light) and which had no significant effect on lens-induced refractive errors, reserpine also affected lens-induced changes in eye growth. At lower doses, the effect was selective for negative lenses (Fig. 4). We found that the individual retinal dopamine levels were very variable among individuals but were correlated in both eyes of an animal; a similar variability was previously found with regard to deprivation myopia. To test a hypothesis raised by Li, Schaeffel, Kohler and Zrenner [(1992) *Visual Neuroscience*, 9, 483–492] that individual dopamine levels might determine the susceptibility to deprivation myopia, refractive errors were correlated with dopamine levels in occluded and untreated eyes of monocularly deprived chickens (Fig. 6). The hypothesis was rejected. Although it has been previously found that the static retinal tissue levels of dopamine are not altered by lens treatment, subtle changes in the ratio of DOPAC to dopamine were detected in the present study. The result indicates that retinal dopamine might be implicated also in lens-induced growth changes. Surprisingly, the changes were in the opposite direction for deprivation and negative lenses although both produce myopia. Currently, there is evidence that deprivation-induced and lens-induced refractive errors in chicks are produced by different mechanisms. However, findings (1), (3) and (5) suggest that there may also be common features. Although it has not yet been resolved how both mechanisms merge to produce the appropriate axial eye growth rates, we propose a scheme (Fig. 7).

Myopia Dopamine Melatonin Lenses Deprivation Chickens

INTRODUCTION

Two different experimental manipulations of visual experience are currently used to induce experimental refractive errors in chickens: first, image degradation by translucent eye occluders and, second, defocusing lenses. Occluders produce variable amounts of myopia ("deprivation myopia") even though the image degradation may be quite moderate (Bartmann & Schaeffel, 1994). In fact, more complete visual deprivation (black eye occluders) is less efficient than minor deteriorations of retinal image quality (Sivak, Barne & Weerheim, 1989; Bartmann & Schaeffel, 1994). The extent of the "deprivation" can be quantified by measuring the modulation transfer functions (MTF) of the occluders. In the present study, we have used the "slightly frosted" type of occluders with the MTF given previously (Bartmann & Schaeffel, 1994) and refer to the induced myopia as 'deprivation myopia'.

Following the initial assumption that deprivation myopia is triggered by exaggerated accommodation

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which was assumed to take place as a result of the degraded retinal image (Wallman, Rosenthal, Adams & Romagnano, 1981; McKanna & Casagrande, 1981), it became clear that the responsible mechanism is, in fact, operating locally in the retina (Wallman, Gottlieb, Rajaram & Fugate-Wentzek, 1987), even without the need of an intact optic nerve connecting the eye to the brain (Raviola and Wiesel, 1985; Troilo, Gottlieb & Wallman, 1987) or after ganglion cell blockade by TTX (Norton, Essinger & McBrien, 1994). Although the visual mechanisms that produce deprivation myopia may not be the only ones guiding refractive development with normal vision, considerable efforts are currently made to understand deprivation myopia. Some evidence has accumulated that dopamine plays a role: its retinal levels are lowered during the day in deprived myopic eyes (monkeys: Iuvone, Tigges, Fernandez & Tigges, 1989; chickens: Stone, Lin, Laties & Iuvone, 1989). Application of an agonist of dopamine, apomorphine, was found to suppress development of deprivation myopia, again both in monkeys (Iuvone, Tigges, Stone, Lambert & Laties, 1991) and chickens (Stone *et al.*, 1989; Rohrer, Spira & Stell, 1993). Continuous light (Bartmann *et al.*, 1994) and 6-hydroxy dopamine (6-OHDA; Li *et al.*, 1992) which both lower retinal dopamine levels, also suppress deprivation myopia in chickens. The effect of dopamine on deprivation-induced eye growth involves a D2 receptor mechanism (Rohrer *et al.*, 1993). Various experiments in chickens have shown that a normal diurnal light cycle is necessary for deprivation myopia to develop (Gottlieb, Nickla & Wallman, 1992; Bartmann *et al.*, 1994) and that the eyes grow normally under a marked diurnal rhythm which is interrupted during development of deprivation myopia (Weiss & Schaeffel, 1993). The results suggest that the diurnal rhythms in the eye play a role in the development of deprivation myopia; the major internal rhythm in the eye is expressed by the retinal dopamine/melatonin system which makes its importance likely in deprivation myopia.

The second procedure of inducing refractive errors is the treatment with defocusing lenses. Lenses produce a fundamentally different kind of image degradation than occluders because their effect (pure defocus) can be cleared by accommodation, at least if weak lenses are used as in the present study [chickens also have negative accommodation (about 4 D; Troilo, Tong & Howland, 1993)]. Despite a clear image, chicken eyes compensate lens-imposed refractive errors by changing their axial eye growth rates (Schaeffel, Glasser & Howland, 1988; Wildsoet & Wallman, 1992; Irving, Sivak & Callender, 1992). There is evidence that the lens-triggered growth control feedback loop is different from the one responsible for deprivation myopia: 6-OHDA suppresses deprivation myopia but not lens-induced refractive errors in chickens (Schaeffel, Hagel, Bartmann, Kohler & Zrenner, 1994a). The same is true for continuous light (Bartmann *et al.*, 1994). Therefore, the relationship of lens-triggered refractive errors to diurnal growth rhythms and to retinal dopamine is unclear and requires additional experiments.

In the current study, we tested whether growth changes produced by defocusing lenses also implicate changes in diurnal growth rhythms of the eye and studied the effects of drugs that interfere with the dopamine/melatonin system on the development of refractive errors. In addition, we tried to answer the question how the large inter-individual variability of deprivation myopia (Wallman & Adams, 1987; Schaeffel & Howland, 1991) can be explained. Retinal dopamine levels exhibit a large inter-individual variability and, as a simple hypothesis, we tested whether they determine the individual susceptibility to deprivation myopia. Finally, we present a scheme incorporating the current understanding of the feedback loops controlling experimental refractive errors in chicks.

MATERIALS AND METHODS

Animals

Male chickens originating from a white leghorn egg strain were obtained from a local hatchery. Two chickens were used to test the immediate effects of intravitreal injections on the ERG responses (Fig. 1). Diurnal growth rhythms with lenses were recorded in 12 chickens, 6 with bilateral negative lenses and 6 with bilateral positive lenses [Fig. 2(A)]. The effect of reserpine on deprivation myopia was tested on 4 (0.2 μg), 8 (2 μg), and 8 (20 μg) chickens, respectively [Fig. 3(A)]. Electroretinograms after reserpine application were recorded in 18 chickens, including controls [Fig. 3(C)]. Lens-induced refractive errors were studied after reserpine application (2 μg) in 13 chickens wearing +4 D lenses in one eye and −4 D lenses in the other eye (see Results), and in 36 chickens with +7 D lens or −8 D lens in one eye and no lens in the other eye (Fig. 4). The effects of melatonin were tested in 4 (200 μg), 5 (500 μg), 5 (1000 μg), 3 (2000 μg) chickens, respectively (Table 1, Fig. 5). Fourteen chickens were used to test 5,7-DHT (Table 1, Fig. 5). Sulpiride was studied in 4 (10 μg), 9 (100 μg), 7 (200 μg), and 6 (400 μg) chicks, respectively (Table 1). The Schering antagonist Sch23390 was tested in 3 (1 μg), 6 (10 μg), and 6 (20 μg) chickens (Table 1, Fig. 5). Finally, 18 chickens were involved in the studies of the retinal DOPAC to dopamine ratio after 2 days of lens or occluder treatment (no figure). Thirteen chicks provided the interocular correlation of retinal dopamine levels with normal visual experience [Fig. 6(A)]. In 19 chickens, it was tested whether individual retinal dopamine levels could serve as a predictor for the amount of deprivation myopia [Fig. 6(B, C)]. In total, 223 chickens were used in the present study. The treatment of the chickens was approved by the University commission of animal welfare (AK 2/90 and AK 2/91) and was in accordance with the ARVO resolution for care and use of laboratory animals.

Optical techniques and A-scan ultrasound

Refractive state was measured by an improved version of infrared photoretinoscopy in which the slope of the brightness profile in the pupil was automatically measured by a video digitizer board and then converted into refractive error (Schaeffel, Hagel, Eikermann &

Collett, 1994b). The refraction technique was very valuable because natural accommodation could be quantitatively evaluated. Axial length was measured by A-scan ultrasonography as described earlier (Schaeffel & Howland, 1991). The term "axial length" used in the text refers to the distance from the corneal apex to the vitreo–retinal interface and is the sum of anterior chamber depth, lens thickness and vitreous chamber depth (Table 1); choroidal thickness could not be directly measured. Anterior chamber depth and lens thickness were also measured and are given in Table 1. Our techniques had the advantage that they were not invasive and did not require anesthesia.

ERG recordings

A detailed description of the recording apparatus has been given previously (Schaeffel, Rohrer, Lemmer & Zrenner, 1991). We used a special chicken contact lens electrode made by the Medical workshop, Gronningen, Holland. To measure the spectral sensitivity curves [Fig. 3(C)], a 60 μV criterion amplitude was chosen (measured from the trough of the *a*-wave to the maximum of the *b*-wave) to determine the relative sensitivity which was then plotted as the negative of the log quanta/μm^2* sec necessary to achieve the criterion response.

Immunohistochemistry

Transversal frozen section were stained using a monoclonal antibody against tyrosine hydroxylase (Incstar, Stillwater, Minn.) which was visualized using FITC. Serotonin was labelled using a polyclonal antibody raised in rabbit (Incstar) and visualized using TRITC.

Intravitreal injections

50 μl of vehicle containing various amounts of the tested drugs (as indicated Table 1) were very gently and slowly injected with a 0.4 × 20 mm syringe cannula into the vitreous of the eyes through skin and sclera close to the margin of the upper orbit under general ether anesthesia. The fellow eye received the same amount of vehicle. The vehicle consisted out of saline and 0.1% ascorbate except for melatonin and reserpine which were pre-dissolved in dimethylsulfoxide (DMSO, pure agent) and then diluted in saline to an end concentration of 30% DMSO. All drugs were obtained from Sigma. Each animal was injected only once. Chickens were foraging normally 10 min subsequent to the injection. In two chickens, electroretinograms were recorded during the injection procedure to test whether retinal function deficits occurred as a result of the transient increase in intraocular pressure. During these experiments, the chickens remained in the recording apparatus with the contact lens electrode in place. The ERG-amplitude dropped during the time when the injection needle penetrated the ocular coat but it recovered to its pre-injection value within about 10 min (Fig. 1). In addition, the fact that vehicle injected eyes generally responded to deprivation with a similar amount of myopia as the non-injected eyes (see Table 1 for exceptions due to shorter periods of occlusion) was considered as an indication that they were intact.

Experimental procedures

From the day of hatching, white leghorn chickens were kept under a 12 hr light:dark cycle (light from 8 a.m. to 8 p.m.). The cages were illuminated from above by a single 60 W light bulb which resulted in illuminance levels

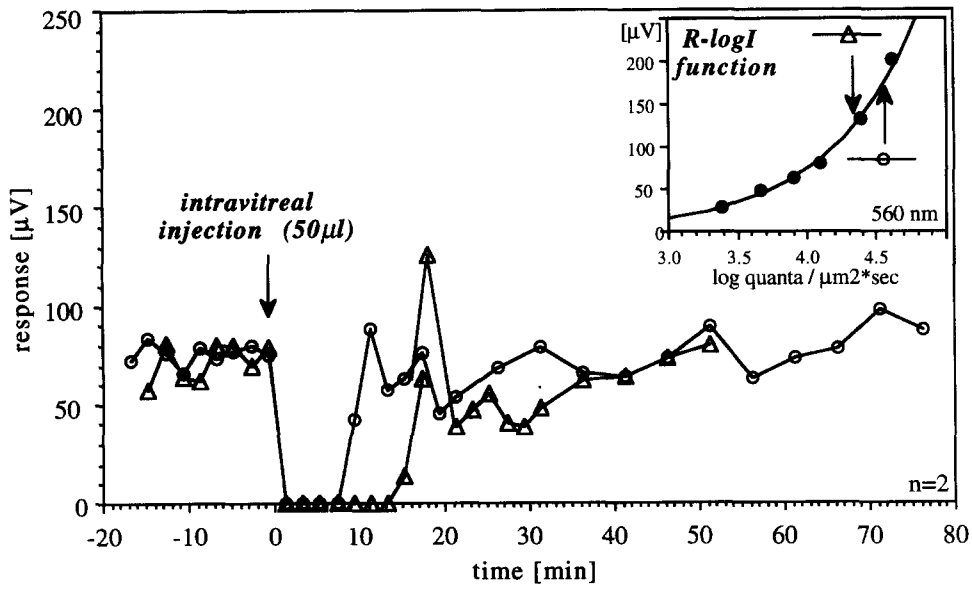

FIGURE 1. Effect of intravitreal vehicle injections on the amplitude of the electroretinogram. The light intensities used for stimulation were chosen to stimulate in the linear range of the response (R–log I) function (see inset). They were 4.38 log quanta/μm^2 sec for chick 1 (see arrow in the inset △) and 4.62 log quanta/μm^2 sec for chick 2 (arrow, ○). After intravitreal injection, no ERG was measurable at the respective intensities; responses could only be elicited at higher intensities (not shown). The sensitivity recovered to its pre-injection value after 10–15 min.

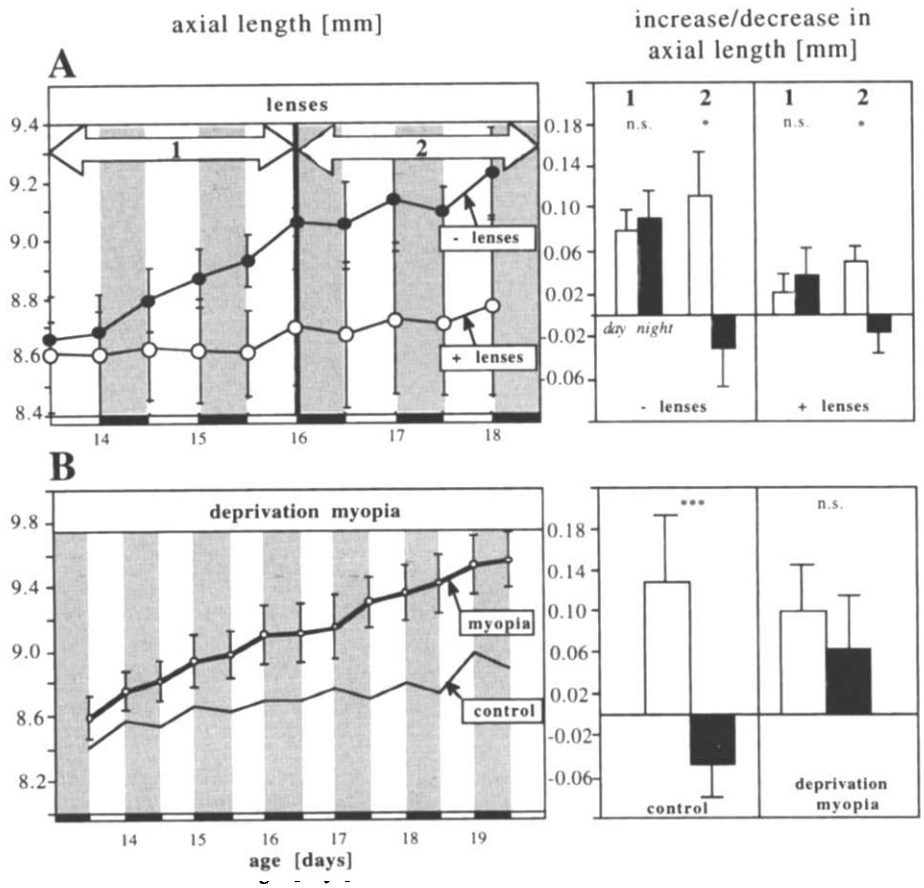

FIGURE 2. Diurnal growth rhythms in chicken eyes during spectacle lens treatment (A) and during occluder treatment (B). On the left, the average axial lengths are plotted vs age in days. On the right, the average growth rates at day (white bars) and at night (black bars) are shown. (A) During lens treatment, the growth rhythms are changed and become similar to the ones measured during deprivation. However, the abnormal growth patterns persist only until the compensation of the lenses is complete (window "1") and they return to normal [compare to "control" in (B)] thereafter (window "2"). (B) Growth rhythms during deprivation shown for comparison (data replotted from Weiss & Schaeffel, 1993).

ranging from 1000 to 3000 lx, depending on direction of measurement. No additional room light was present and the animal facilities had no access to day light. The experimental protocol was similar in all cases: 4–9 chickens were raised for each data point. Measurements (refraction and A-scan ultrasound) started at day 8. Intravitreal injections took place on day 10 [as indicated in Fig. 3(A) by arrows]. On day 10, chickens were fitted with translucent occluders over *both* eyes, except for the "non-injected controls" shown in Table 1. Occluders (Li *et al.*, 1992) were glued to the feathers around the eye with instant glue under light ether anesthesia. They were removed on day 15 (melatonin experiments at 500 to 1000 μg, sulpiride experiments, Sch23390 experiment with 10 μg, see Table 1) or on day 18 (all other experiments, see Table 1). The occluders had a frontal gap (about 15 deg wide) to permit normal foraging even with binocular application. As can be seen from Table 1, similar amounts of myopia developed on-axis in chicks that wore whole-field occluders in one eye (-7.0 ± 3.8 D, $n=15$) and in chicks that were binocularly occluded as described above (see vehicle-injected control eyes at day 18 in the drug experiments, Table 1). The advantage of binocular occlusion is that, due to the high correlation of deprivation myopia in both eyes of non-injected chicks (Schaeffel & Howland, 1991), differences in injected eyes can be attributed to the action of the applied drugs on deprivation myopia rather than to its genetical variability. One could also employ another protocol, in which both eyes are drug-injected but one eye is occluded only. To obtain significant results, larger individual numbers are necessary due to the natural variability of deprivation myopia. In addition, in eyes with normal vision, axial growth and refractions are much more stable since they still grow under "closed loop conditions". Any visually-guided feedback loop will keep the refractions in the optimal range, as long as it can operate. We therefore preferred our present protocol to study deprivation myopia although one disadvantage is that the "seeing" part of the retina may differ in content of biogenic amines and may therefore "contaminate" our HLPC measurements.

Lenses were either fitted in front of both eyes ($+4/-4$ D) or were monocularly applied ($+7$ D or -8 D); they were attached to small leather hoods (Schaeffel *et al.*, 1988). Occluders were left in place for 5–8 days, whereas lenses were removed already after 4 days because the maximum refractive difference was reached by then (Fig. 4, controls).

Measurement of biogenic amines

Biogenic amines were measured by HPLC and were plotted as ng biogenic amine per mg protein. Protein measurement was done by the method of Lowry, Rosebrough, Farr and Randall (1965) with bovine serum albumin as standard. The High Pressure Liquid Chromatography System (HPLC) is described in detail in a previous paper (Bartmann *et al.*, 1994).

Preparation of samples

Preparation of the retinae for HPLC measurements was performed immediately after the removal of the occluders or lenses. To reduce effects of inter-batch and inter-individual variability in the measurements of retinal catecholamine levels (see Table 1), retinal tissue levels were compared in differently treated eyes from animals of the same batches. Chickens were deeply anesthetized by ether and decapitated. Since it is known that DA levels are light dependent (Parkinson & Rando, 1983; Stone *et al.*, 1989) and serotonin levels undergo diurnal variations (Siuciak, Gamache & Dubocovich, 1992), all preparations were done at noon. Both retinae were dissected out without pigment epithelium within a few minutes under a preparation buffer (phosphate, pH 7.4) and each retina was collected in an ice-cooled homogenizer (Braun, Melsungen, Germany) containing 1 ml of eluens with 10 ng of the internal standard dihydroxybenzylamine (DHBA). Even though catecholamines and indolamines proved to be stable in this eluens for several hours at room temperature, the samples were kept on ice during the whole procedure. The retinae were then homogenized by a motor driven pestle for 30 sec. 50 μl of the solution were separated for subsequent protein measurement. The remaining homogenate was centrifuged for 2 min at 14,000 rpm (not refrigerated) and the supernatant filtered through a 0.2 μg sample filter (Schleicher and Schuell, Dassel, Germany) and stored at -70°C until use. Prior to the analysis, the samples were centrifuged again for 30 sec and then loaded into the manual injector of the HPLC set-up.

Statistics

All data are given as mean values from 4–9 chickens $\pm$ SD, except for Fig. 2, in which standard errors are given in the upper right graph. Whenever differences between treated and untreated (left/right) eyes from a group of animals are plotted, Student's paired t-test was used to calculate significances. If two groups of animals were compared, unpaired t-tests were employed. The group sizes were variable for technical reasons: due to limitations in the allowed group sizes and to speed up the experiments, groups were divided in some cases and the subgroups were differently treated (Table 1). It is clear that the statistical analysis would be more homogeneous if equal numbers of individuals were used for all experiments. However, in our data (Table 1), most of the significant differences were already found in groups of only four chickens, and it appears that none of the remaining groups would have shown significant differences if their numbers were increased.

RESULTS

(1) Relation of lens-induced growth changes to diurnal growth rhythms in the eye

It was previously found that deprivation myopia developed as a result of a disturbance of diurnal growth rhythms in the eye (data replotted from Weiss & Schaeffel, 1993, Fig. 2B). We have now measured diurnal changes in axial length also in eyes while they were compensating lens-imposed defocus. The result was unexpected: also lenses changed diurnal growth rhythms [Fig. 2(A)]. Although compensation of the weak lenses used ($+4/-4$ D) took only 2 days [see times frame "1" in Fig. 2(A)], the growth rhythms were clearly different from those in eyes with normal visual experience [see graphs in the right panel of Fig. 2(B)]. In contrast to deprived eyes, the lens-treated eyes returned to their normal growth rhythms even with the lenses in place as soon as the compensation of induced refractive errors was complete [time frame "2" in Fig. 2(A)]. At the same time, growth rates at day and night were significantly different, similar to eyes with normal vision, for both positive- and negative lens treated eyes ($n=6$ for bilateral $+4$ D lenses and $n=6$ for bilateral -4 D lenses, $P<0.05$ in both cases). The result suggests that, at some level, both lens-induced and deprivation-induced growth changes implicate disturbances of diurnal rhythms in the eye and, therefore, may be partly initiated by a common mechanism.

(2) Inhibition of deprivation myopia by reserpine

So far, we had used two procedures to suppress deprivation myopia: intravitreal injection of 6-OHDA (Schaeffel *et al.*, 1994a) and exposure to continuous light (Bartmann *et al.*, 1994). In both cases, retinal catecholamine levels were lowered and, in the case of continuous light, also serotonin levels dropped. In the present study, we tested reserpine which lowered both dopamine and serotonin levels [Fig. 3(B)]. Reserpine was found to be extremely efficient in blocking deprivation myopia [Fig. 3(A)]. It was found effective at doses of 1/100 of the necessary dose of 6-OHDA. Suppression of deprivation myopia was significant at 0.2 μg (axial lengths: vehicle-injected 9.79 ± 0.05 mm, reserpine-injected 9.46 ± 0.18 mm, $P<0.05$). At this dose, no changes could be seen in HPLC measurements of retinal biogenic amines [Fig. 3(B)]. At doses of 2 μg or more, reserpine reduced both the levels of dopamine and serotonin to less than 30% of their initial values [Fig. 3(B)] and suppressed deprivation myopia very efficiently (reserpine-injected eyes were 0.54 mm shorter than vehicle-injected eyes, $P<0.0001$). At 20 μg, the inhibition was also significant [Fig. 3(A), Table 1]. To test whether the retina was still intact after reserpine application, we recorded ERGs for the different doses 5 days after the injection [Fig. 3(C)]. Strikingly, even at the highest doses, there were no changes detectable in spectral sensitivity

as determined from a 60 μV criterion response (difference from the trough of the *a*-wave to the peak of the *b*-wave). We did not analyse the ratio of *a*-wave to *b*-wave amplitudes in the original traces; an increase of *a*-wave amplitude with a concomitant decline of *b*-wave amplitude could have produced similar criterion responses. Finally, in retinae of eyes injected with 20 μg of reserpine, tyrosine hydroxylase and serotonin immunoreactive cells were labelled immunohistochemically. The most striking observation was that tyrosine hydroxylase immunoreactivity was unchanged after the reserpine injection [Fig. 3(D)] even though the HPLC measurements had shown that the dopamine content had dropped to 20–30% of the control values [Fig. 3(B)]. Serotonergic cells had virtually disappeared [Fig. 3(D)].

(3) Inhibition of lens-induced refractive errors by reserpine

In contrast to 6-OHDA or continuous light which both had no effect on lens-induced refractive errors (Schaeffel *et al.*, 1994a; Bartmann *et al.*, 1994), we found that lens experiments did *not* work equally well with reserpine. In previous experiments, after four days of lens treatment (+4/−4 D; from day 12–16), non-injected control eyes showed a difference in axial length of 0.41 ± 0.27 mm ($n=4$, data from Bartmann *et al.*, 1994). A single intravitreal injection of 200 μg 6-OHDA did not change the magnitude of the effect of the lenses after 4 days of treatment (from day 13–17; difference in axial lengths: 0.38 ± 0.18 mm, $n=10$, data from Schaeffel *et al.*, 1994). In contrast, at a dose of 2 μg reserpine, the difference in axial length between plus and minus lens-treated eyes was

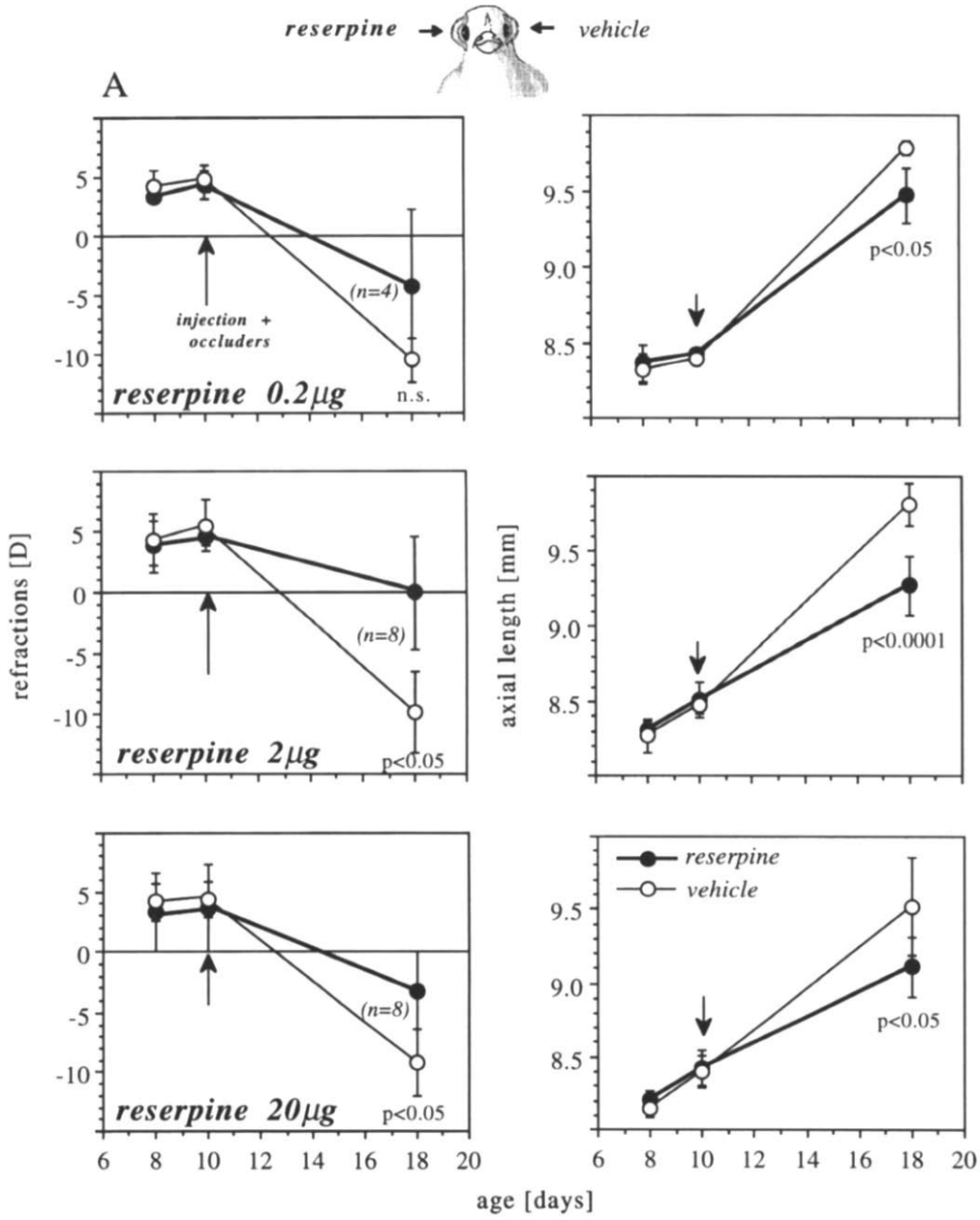

FIGURE 3(A). *Caption on p. 1255.*

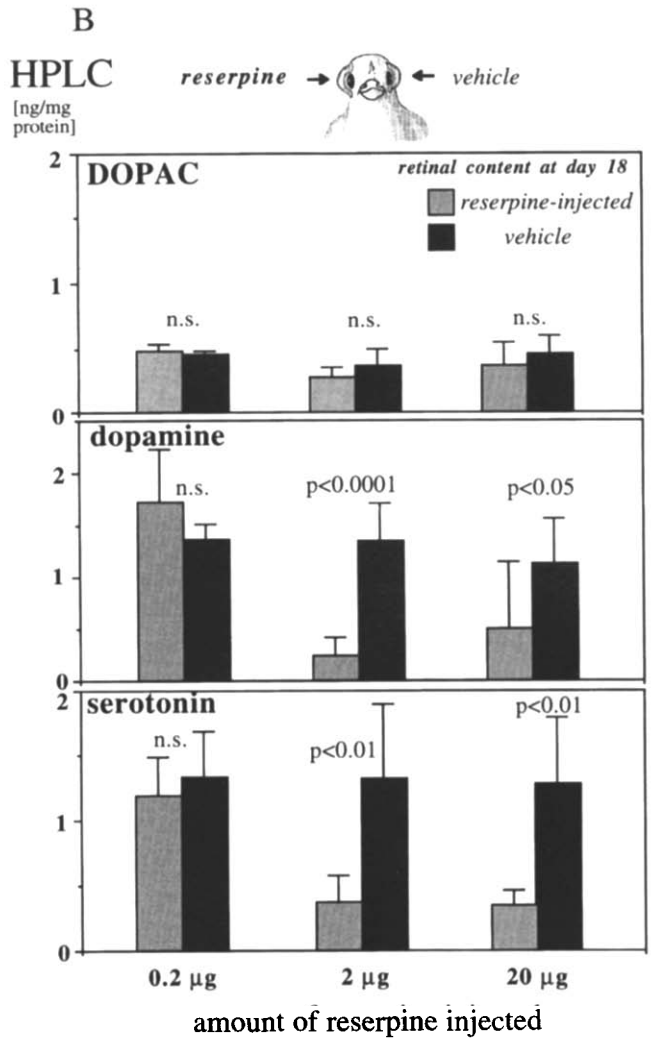

FIGURE 3(B). *Caption on p. 1255.*

reduced to 0.15 ± 0.12 mm ($n = 13$). The lenses were significantly less efficient than in the 6-OHDA-treated eyes (d.f. = 21, $T = 3.5$, $P < 0.01$, unpaired t-test). To find out whether the suppression of the lens effects were restricted to lenses of one sign, we raised chicks with lenses only on one side (+7 D vs no lens and −8 D vs no lens). Strikingly, at 2 µg, positive lenses were equally efficient than in non-injected controls but negative lenses had no longer an effect on refractive development (Fig. 4, compare refraction data marked by open arrows). Even at 20 µg, there was still some effect of the positive lenses although the differences in axial lengths had disappeared for both types of lenses.

(4) Enhancement of deprivation myopia by melatonin, 5,7-DHT, sulpiride and Sch23390

Melatonin. Lowering retinal dopamine levels by either continuous light, 6-OHDA, or reserpine suppressed deprivation myopia. With normal vision, retinal dopamine and melatonin levels are inversely correlated (Nowak, Kazula & Golembiowska, 1992; Rudolf, Vivien-Roel, Pevet, Kempf & Wioland, 1992) due to the D2-receptor mediated inhibition of *N*-acetyl-transferase (Iuvone & Besharse, 1986) which catalyzes the synthesis of a melatonin precursor from serotonin. In addition, tryptophan hydroxylase activity is diurnally modulated (Thomas, Tigges & Iuvone, 1993). Despite the short life time of the injected melatonin in the vitreous, we expected that it would cause a transient drop in dopamine and, therefore, reduce deprivation myopia. With a single intravitreal injection on day 10 of either 200 or 500 µg and subsequent occlusion, no significant effects on deprivation myopia were observed (Table 1). However, at 1000 µg, deprivation myopia was enhanced (Table 1). At this dose, the diurnal activity pattern of the chickens was disturbed since they slept during the day. At even higher doses (2000 µg, $n = 3$; Fig. 5), melatonin suppressed deprivation myopia (-3.8 ± 1.6 D vs -12.4 ± 1.7 D, $P < 0.05$; axial lengths: 9.22 ± 0.12 mm vs 9.52 ± 0.15 mm, NS). Retinal dopamine levels could only be tested 9 days later at the end of the experiment with 1000 µg (Table 1). No changes were found at this time.

5,7-Dihydroxy-tryptamine (5,7-DHT). Similar to the effect of 6-OHDA on dopaminergic cells, 5,7-DHT depletes serotonergic cells by formations of free radicals and hypoxia (Tabatabaie, Goyal, Blank & Dryhurst, 1993). Although depletion of the cells in the retina that contain most of the serotonin [bipolar and amacrine cells;

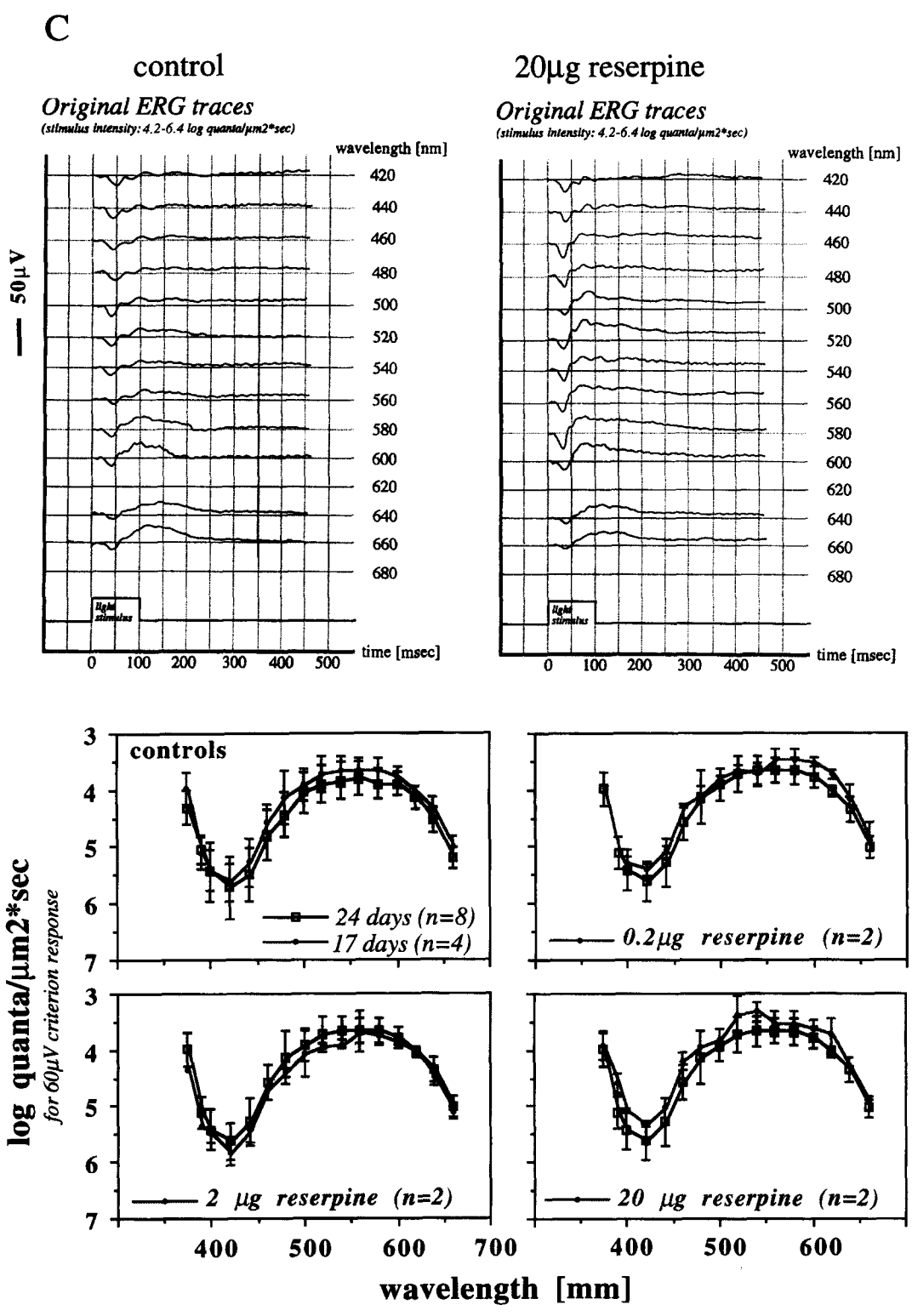

FIGURE 3(C). *Caption on facing page.*

see Fig. 3(D)] does not interrupt the diurnal melatonin cycles in the photoreceptors (Thomas *et al.*, 1993), we assumed that the treatment would have an effect on the diurnal retinal serotonin rhythms (Siuciak *et al.*, 1992) and on deprivation myopia. At a dose of 100 µg, 5,7-DHT had no effect (Table 1). However, at 200 µg, deprivation myopia was significantly enhanced (Table 1). At the same time, retinal serotonin levels were reduced by the treatment with 5,7-DHT (Table 1). No effect was found on retinal dopamine (Table 1).

Sulpiride. Sulpiride is a specific D2 receptor antagonist that can induce an up-regulation of retinal dopamine levels (DaPrada, 1977). We found that sulpiride enhanced deprivation myopia at doses of 100 and 400 µg. At 100 µg (Table 1), sulpiride treated eyes were more myopic than the vehicle injected eyes and were longer (9.84 ± 0.83 mm)

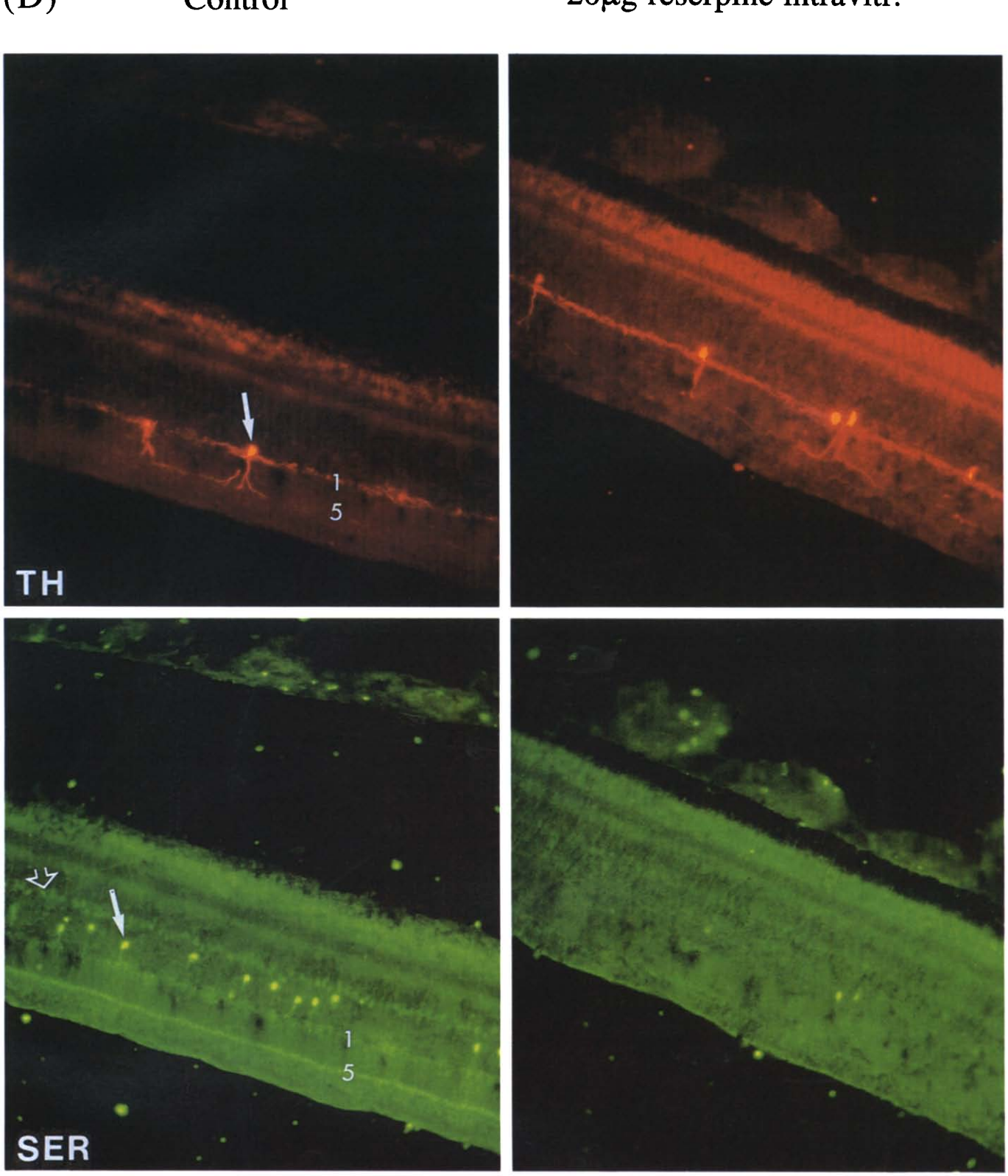

FIGURE 3. Effects of reserpine on deprivation myopia, retinal biogenic amines, electroretinograms and TH- and serotonergic cells in the retina. (A) The treatment protocol is illustrated on the top. Deprivation-induced myopia (left column) and axial elongation of the eye (right column) is suppressed by a single intravitreal injection of reserpine. (B) Retinal biogenic amines were determined after removal of the occluders at day 18. Reserpine depletes both retinal dopamine and serotonin but had no effect on DOPAC levels. (C) Even with the highest dose of reserpine, there were no obvious changes in ERG wave forms 4 days after the reserpine injection (top) or on spectral sensitivity functions (bottom). "Controls" originate from chickens of two different ages to illustrate the normal variability of the recordings. (D) Tyrosinhydroxylase (TH)- and serotonin (SER)-immunoreactive cells stained in the same samples of retinal tissue (photoreceptor side up). Left column: vehicle-injected controls. Filled arrows: amacrine cells, open arrow: bipolar cells. "TH"-cells stratify in sublayer 1 ("1") and sublayer 4–5 ("5") of the inner plexiform layer. "Ser"-cells stratify in sublayer 1 ("1") with an immunoreactive ribbon in sublayer 5 ("5"). Right column: 4 days after intravitreal injection of 20 μg reserpine, TH-immunoreactive cells appear normal whereas SER-immunoreactive cells have disappeared.

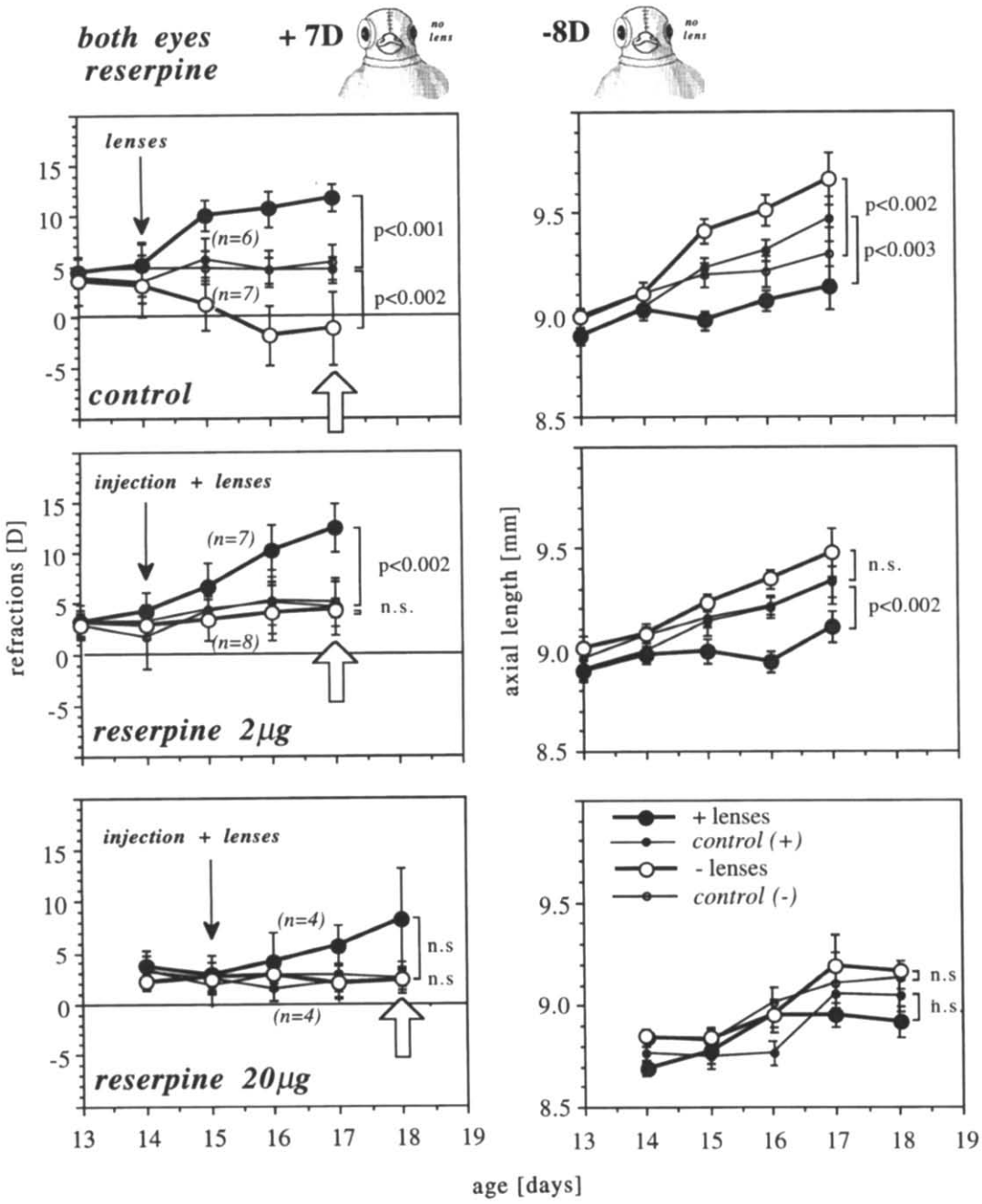

FIGURE 4. Effect of intravitreal reserpine on lens-induced refractive errors. Treatment protocols are illustrated on the top. Refractions are plotted versus age on the left column, axial eye growth is shown on the right column. Note that the effects of negative lenses are blocked at 2 µg but the effects of positive lenses remain unchanged. At 20 µg, also the effects of positive lenses are no longer significant.

than the vehicle injected controls (9.52 ± 0.63 mm, $P < 0.05$). At 200 µg, the sulpiride injected eyes were not significantly more myopic and the axial lengths were not different (Table 1). At 400 µg, the differences were 3.2 D ($n = 7$, $P < 0.01$) and 0.34 mm ($P < 0.01$), respectively. At 10 µg, no effect could be seen on axial lengths but there was a slight reduction in the amount of myopia in the sulpiride treated eyes. Deprivation myopia was generally less in these experiments because the chickens wore their occluders only for 5 days rather than for 8 (see Materials and Methods). Due to some temporary trouble with the HLPC set-up, data could only be collected for the 100 µg injection group. Both serotonin and dopamine levels were raised by sulpiride injections ($P < 0.05$ in both cases, paired t-tests, Table 1).

Sch23390. Finally, a specific antagonist against the D1-receptor, Sch23390, was tested. At 1 µg, Sch23390 had little effect on deprivation myopia (Table 1). Eyes injected with 1 µg showed a tendency towards more myopia although the difference in axial length did not achieve statistical significance (8.95 ± 0.04 mm vs 8.89 ± 0.04 mm, NS). At 10 µg, no significant effect was found either although there was again a tendency towards more myopia (Table 1; axial lengths: 9.32 ± 0.48 mm vs 9.16 ± 0.48 mm, NS). Significance was not achieved due to the large standard deviations (only some animals were

TABLE 1. Effect of intravitreal drugs on deprivation myopia

vehicle drug

applied drug	not-injected controls (15)			
reserpine		0.2µg (4)	2µg (8)	20µg (8)
refractions [D]	2.9±1.6/-7.0±3.8 ***	-10.5±1.9/-4.3±6.6 *	-9.9±3.4/-0.07±4.6 ***	-9.2±2.8/-3.2±4.4 **
ACD [mm]	1.37±0.12/1.39±0.1	1.31±0.07/1.22±0.06	1.34±0.08/1.27±0.12	1.32±0.08/1.3±0.08
lens [mm]	2.27±0.10/2.26±0.10	2.26±0.05/2.21±0.02	2.27±0.09/2.23±0.07	2.1±0.14/2.2±0.13
VC [mm]	5.69±0.11/6.46±0.34 ***	6.22±0.07/6.03±0.26 *	6.2±0.19/5.77±0.2 ***	6.1±0.24/5.84±0.35 *
HPLC [ng/mg prot.] dopamine	0.87±0.63/0.98±0.43	1.09±0.13/1.38±0.39	1.35±0.35/0.24±0.16***	1.33±0.56/0.37±0.2 **
HPLC [ng/mg prot.] serotonin	1.17±0.77/1.56±1.32	1.34±0.35/1.2±0.29	1.62±0.6/0.43±0.14 **	0.91±0.34/0.4±0.51 *
HPLC [ng/mg prot.] DOPAC/DA	0.61±0.19/0.53±0.27	0.34±0.07/0.43±0.08	0.33±0.12/2.14±1.8 *	0.4±0.19/1.25±0.99
5,7-DHT			100µg (7)	200µg (7)
refractions			-7.4±4.13/-7.3±4.8	-7.0±2.9/-11.6±1.7 **
ACD			1.22±0.11/1.27±0.12	1.32±0.1/1.36±0.07
lens			2.23±0.08/2.2±0.09	2.27±0.14/2.34±0.2
VC			6.49±0.33/6.51±0.25	5.99±0.38/6.1±0.4 *
HPLC dopamine			0.94±0.29/0.86±0.12	1.2±0.64/1.14±0.53
HPLC serotonin			0.98±0.28/0.25±0.25 **	1.2±0.79/0.52±0.39 *
HPLC DOPAC/DA			0.35±0.08/0.41±0.17	0.26±0.1/0.26±0.08
6-OHDA+			125µg (4)	200µg (5)
refractions			-9.9±2.2/-4.7±4.6 *	-9.1±1.2/-0.1±1.2 ***
ACD			1.63±0.26/1.55±0.32	1.5±0.24/1.42±0.37
lens			2.31±0.06/2.17±0.03 *	2.2±0.08/2.13±0.05
VC			6.29±0.1/6.20±0.15	6.18±0.12/5.8±0.04**
HPLC dopamine			0.89±0.16/0.63±0.29	0.8±0.28/0.46±0.28 **
HPLC serotonin			0.98±0.03/0.94±0.1	1.08±0.26/0.7±0.27
HPLC DOPAC/DA			0.27±0.13/0.19±0.07	0.3±0.06/0.37±0.22
sulpiride		10µg (4)	100µg (9)	200µg (7)	400µg (6)
refractions		-3.1±2.6/-0.5±1.34 *	-5.0+5.2/-7.3+6.1 *	-1.5±1.8/-2.5±2.5	-4.5±4.2/-7.8±5.9 *
ACD		1.43±0.14/1.47±0.11	1.33±0.13/1.39±0.06	1.2±0.08/1.29±0.1	1.3±0.08/1.47±0.07 **
lens		2.30±0.02/2.3±0.05	2.27±0.08/2.26±0.09	2.32±0.18/2.32±0.3	2.25±0.08/2.27±0.12
VC		5.92±0.28/5.9±0.34	5.87±0.26/6.02±0.38	5.63±0.14/5.6±0.16	5.54±0.06/5.74±0.19 *
HPLC dopamine		*n.d.*	0.77±0.24/1.15±0.38 *	*n.d.*	*n.d.*
HPLC serotonin			0.94±0.34/1.35±0.29 *		
HPLC DOPAC/DA			0.50±0.18/0.43±0.08		
melatonin			200µg (4)	500µg (5)	1000µg (5)
refractions			-7.3±4.3/-6.5±4.6	-4.5±4.9/-7.3±4.1 *	-4.44±4.8/-8.0±2.2 *
ACD			1.29±0.05/1.28±0.08	1.17±0.09/1.25±0.11 *	1.28±0.05/1.34±0.04
lens			2.31±0.1/2.36±0.08	2.3±0.06/2.28±0.07	2.26±0.09/2.37±0.11
VC			6.3±0.26/6.22±0.05	5.54±0.12/5.6±0.16	5.64±0.31/5.91±0.02 *
HPLC dopamine			*n.d.*	*n.d.*	1.16±0.13/1.25±0.34
HPLC serotonin					1.37±0.16/1.21±0.36
HPLC DOPAC/DA					0.27±0.16/0.42±0.21
SCH23390		1µg (3)	10µg (6)	20µg (6)	
refractions		-4.75±3.7/-7.1±3.6	-3.6±4.1/-5.1±5.27	-9.15±4.11/0.62±4.8 **	
ACD		*only axial length*	1.19±0.09/1.30±0.18	1.33±0.05/1.24±0.05**	
lens		*determined*	2.3±0.04/2.27±0.06	2.23±0.09/2.3±0.4	
VC		*(see Fig. 5)*	5.67±0.15/5.79±0.31	5.76±0.32/5.5±0.2 *	
HPLC dopamine		*n.d.*	*n.d.*	*n.d.*	
HPLC serotonin					
HPLC DOPAC/DA					

ACD: anterior chamber depth
lens: lens thickness
VC : vitreous chamber depth

paired t-test: * p<0.05
** p<0.01
*** p<0.001
no statement: not significant

Vitreous chamber depths data that are printed on grey background originate from chickens that were occluded for only 5 days and were, therefore, less myopic and had less deep vitreous chambers ($P<0.01$) than the "not-injected controls" which were occluded for 8 days. The other vehicle-injected deprived eyes did not differ significantly from non-injected deprived eyes. Treatment protocols are illustrated on the top of the table. (+: data from Schaeffel *et al.*, 1994a.)

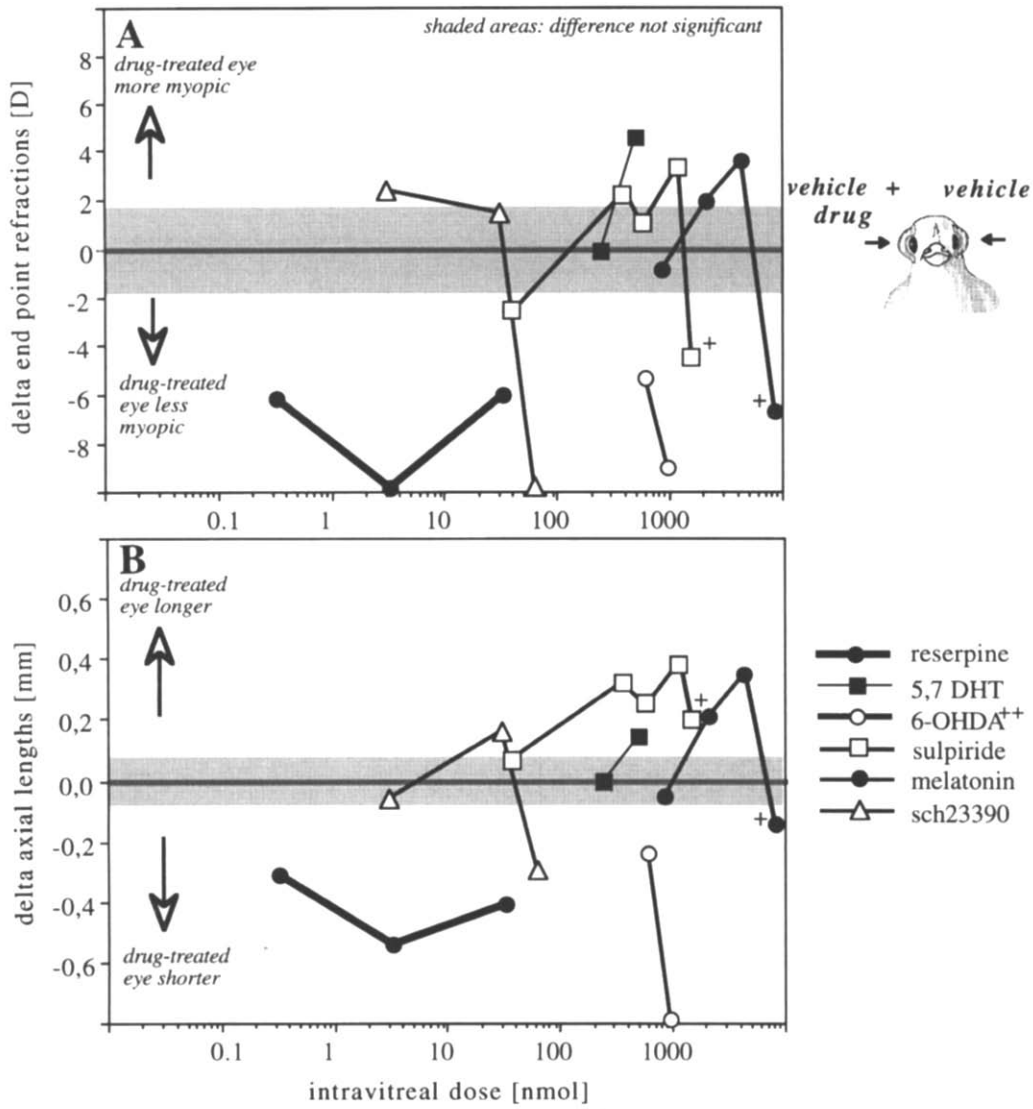

FIGURE 5. Comparison of the relative potency of the drugs used. Interocular differences in refractions (A) and axial lengths (B) with different drugs after 8 days of occlusion (reserpine, 5,7-DHT, 6-OHDA) or after 5 days of occlusion (sulpiride, melatonin, Sch23390) are plotted against molar doses. Note that reserpine, 6-OHDA, and Sch23390 block deprivation myopia, whereas 5,7-DHT, sulpiride and melatonin can enhance it. (+data not in Table 1; + +data from Schaeffel *et al.*, 1994a.)

more myopic in the Sch23390 treated eyes). Finally, at even higher doses (20 μg), Sch23390 blocked deprivation myopia (Table 1) and inhibited deprivation-induced axial eye growth (9.04 ± 0.27 mm vs 9.33 ± 0.35 mm).

To facilitate comparisons of the relative potency of the different drugs in modifying deprivation myopia after a single intravitreal injection, interocular differences between both occluded eyes, one vehicle-injected and the other drug-injected, are plotted for the different molar doses in Fig. 5. It is clear that relative potencies of the drugs are determined by a number of factors that were not separately addressed (like diffusion constants in the vitreous and in the retinal tissue, permeability of the inner limiting membrane, locus of action, half life time, and receptor dissociation constants and metabolisation). Because these factors were not known, the local tissue levels could not be determined. Another factor that was not directly measured was the extent of diffusion of the drug into the control eye. However, since occluded and vehicle-injected fellow eyes in the drug experiments did not differ from the occluded eyes of not-injected controls (except for eyes that were occluded for only 5 days, Table 1), cross-talk between both eyes was not considered to be a major factor.

(5) Inter-ocular correlations of retinal dopamine levels and variability of deprivation myopia

Since enhancement or suppression of deprivation myopia seems to be linked to changes in retinal dopamine levels, it was hypothesized by Li *et al.* (1992) that the "gains" by which the visual experience (in this case, deprivation) is translated into accelerated axial eye growth is determined by individual dopamine levels. We found that the day time retinal dopamine levels were variable among animals with normal visual experience but were highly correlated in both eyes [$r = 0.89$, $n = 13$, $P < 0.001$; Fig. 6(A)]. However, neither in the occluded myopic eye [Fig. 6(B)] nor in the untreated fellow eye [Fig. 6(C)] were there any correlations detectable between amount of myopia and individual dopamine level. The

hypothesis raised by Li *et al.* (1992) must therefore be rejected.

(6) Dopamine turn-over and experimental refractive errors

We have previously found that the overall retinal dopamine levels were not changed during compensation of lens-imposed refractive errors (Bartmann *et al.*, 1994). This was also true for the current study. However, despite of the large inter-group variability in DOPAC/DA ratios (see Table 1), a comparison of both eyes treated with lenses of different sign in 9 chickens showed that, after 2 days of lens treatment, the ratio of DOPAC to dopamine (DA) was significantly ($P<0.05$) changed in positive vs negative lens treated eyes: positive lenses: DOPAC/DA: 0.32 ± 0.08: negative lenses: DOPAC/DA: 0.39 ± 0.08. In a second experiment on 9 chickens, we tested whether axial elongation was paralleled by the same change in the DOPAC to DA ratio, with no regard of whether it was initiated by deprivation or by negative lenses. Strikingly, the expected result was not found: in occluded eyes, after 2 days of treatment, the ratio of DOPAC to DA was 0.24 ± 0.08, and in the untreated control eye it was 0.29 ± 0.08 ($P<0.01$). Therefore, the two manipulations producing axial elongation produced changes of different sign in the ratio of DOPAC to DA: with negative lenses, it increased with respect to positive lenses, and for deprivation it was reduced with respect to controls.

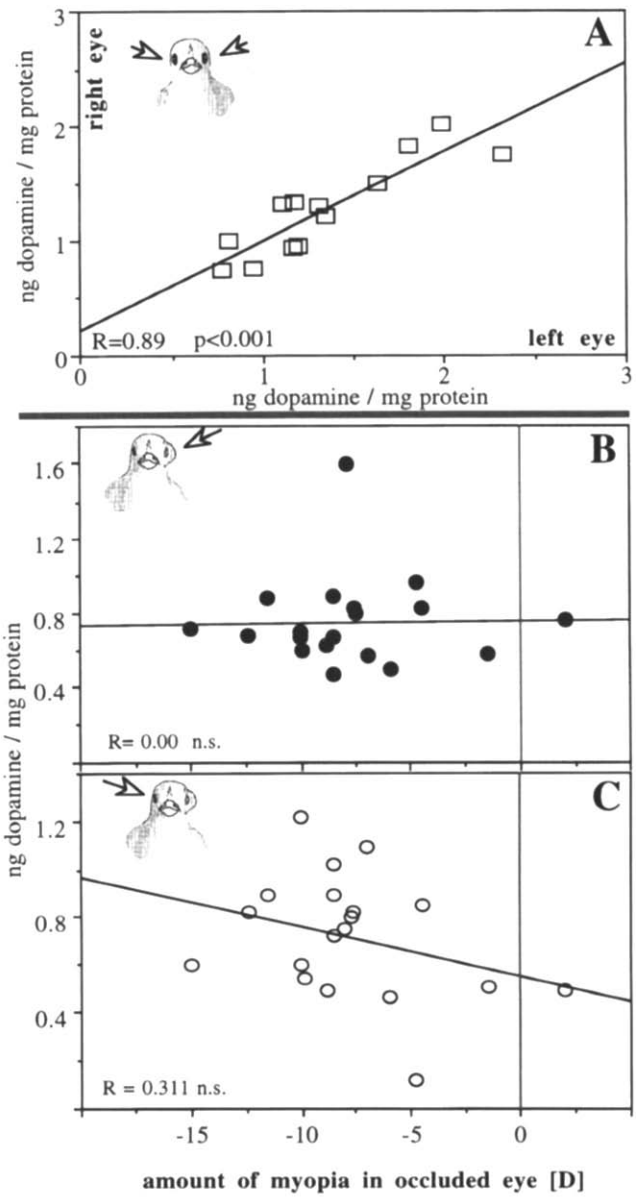

FIGURE 6. Relationship of individual retinal dopamine levels to the variability of deprivation myopia. (A) In chickens with normal visual experience, dopamine levels in the right eye (ordinate) and the left eye (abscissa) are highly correlated ($P<0.001$). (B) Dopamine levels in the occluded eyes (see inserted sketch) do not correlate with the amount of myopia. (C) Dopamine levels in the untreated fellow eye are no predictors of myopia either.

DISCUSSION

We have found that (1) both deprivation-induced and lens-induced growth changes in the chicken eye implicate changes in diurnal rhythms of choroidal thickness and/or axial eye growth, (2) reserpine lowers both retinal dopamine and serotonin and suppresses deprivation myopia very effectively, (3) reserpine can selectively suppress negative lens-induced axial elongation, (4) 5,7-DHT, sulpiride, melatonin and Sch23390 can enhance deprivation myopia, (5) the absolute retinal dopamine levels are no predictors of individual susceptibility to deprivation myopia, and (6) despite of evidence for two independent mechanisms for deprivation-induced and lens-induced growth changes both implicate changes in the retinal dopamine metabolism.

Integrity of visual function after intravitreal injections

Intravitreal injections produce a transient increase in intraocular pressure and it is necessary to consider its effect on visual function. In tree shrews, punctation of the ocular cavity *per se* reduces deprivation myopia (Norton, 1990), indicating that either the increase in pressure or the release of agents during the penetration of the ocular coat is important. Chicken eyes are less sensitive to a single or even several (Rohrer *et al.*, 1993; McBrien, Moghaddam & Reeder, 1993) punctation(s). Our "on-line" ERG recordings during intravitreal injections of 50 μl (Fig. 1) show that the increase in intraocular pressure had only a transient effect on *b*-wave amplitudes and this effect was fully reversible within about 10 to 15 min. An even better argument for preserved retinal function is that, at least for single vehicle injections as in the present study, the injected eyes developed similar amounts of myopia as non-injected eyes given that they were occluded for the same period of time (Table 1).

Another point that needs attention is that some neurotoxins have permanent effects (reserpine, 6-OHDA) whereas other drugs may be effective only for short periods of time. It is clear that, with single intravitreal injections, the tissue levels effective to change visually-induced eye growth are reached only for a few hours. Because deprivation myopia is the result of an "open loop condition" of the feedback loop(s) for visual control of eye growth, we assume that even transient changes in the "gain(s)" of the responsible mechanism(s) show up as

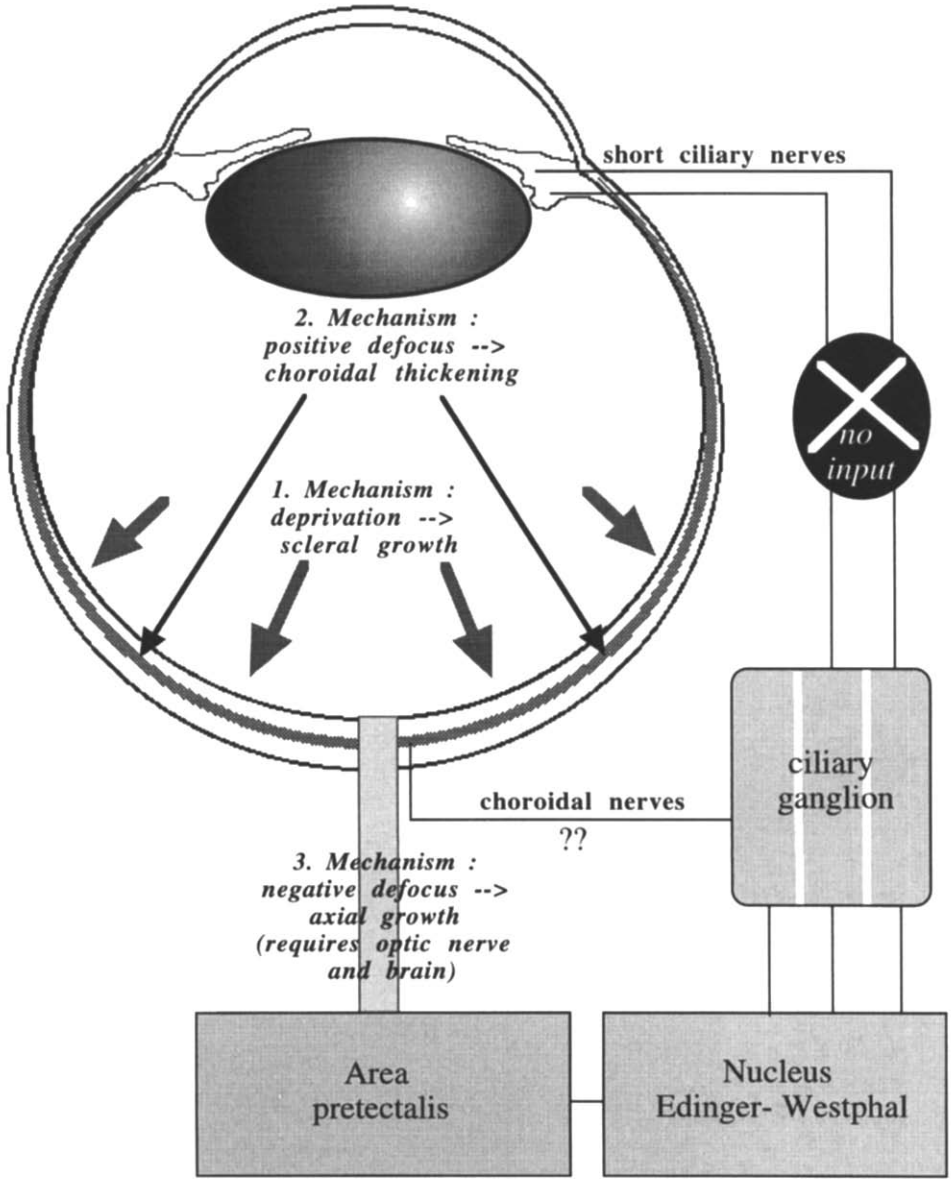

FIGURE 7. Scheme incorporating three mechanisms for visual control of eye growth in chicks (for details see text).

differences in refraction and eye growth at the end of the experiments.

Visual function after drug applications

Development of deprivation myopia does not require intact ganglion cells in chicks (Wildsoet & Pettigrew, 1988a) but the photoreceptors (Oishi & Lauber, 1988) and RPE (Shih, Fitzgerald & Reiner, 1993a) and probably some proximal elements in the retina must be intact. It is an important question whether the applied drugs preserve "normal" visual function because even blinded eyes continue growing normally for a while solely based on their genetical program. They cannot provide information on the function of visually-triggered growth mechanisms. Electroretinograms can help to detect functional deficits in the retina since defects show up in a reduction in spectral sensitivity over the whole spectral range (6-OHDA: Li *et al.*, 1992). More subtle changes may go unnoticed if only spectral sensitivity is analysed. In the present study, even significant reductions in the levels of biogenic amines after reserpine application did not show up in the spectral sensitivity curves [Fig. 3(C)]. The lack of a difference could be explained from the statement of Sato, Yoneyama, Kim and Suzuki (1987) that direct dopamine application had no effect on *a*-, *b*-, or *d*-waves but selectively augmented the *c*-wave. The *c*-wave was not measured in the present study. Other authors have found a reduction of *b*-wave amplitudes after application of an inhibitor (D,L-α-monofluoromethyl dopa, MFMD) of the key enzyme of catecholamine biosynthesis, tyrosin hydroxylase (Rudolf, Wioland, Kempf & Bonaventure, 1990). Therefore, although the original ERG traces were very similar in vehicle- and reserpine-injected eyes [Fig. 3(C)], a more extended analysis of the individual *a*- and *b*-waves might have shown differences.

It is striking that all drugs, including those that enhanced deprivation myopia at lower doses, have finally a supressive effect at higher doses (Fig. 5):

sulpiride > 400 μg, melatonin > 1000 μg and Sch23390 > 20 μg. Sch23390 is a D1-receptor antagonist that is 500 times more potent than sulpiride (Mattingley, Rowlett, Graft & Hatton, 1991). The fact that it had little effect on deprivation myopia at low doses and only blocked it at high doses is in line with the conclusion of Rohrer *et al.* (1993) that deprivation myopia is mainly mediated by a D2 receptor mechanism and supports the idea that the blockade at high doses is unspecific. Similarly, kainate was found to enhance eye growth at lower doses but suppresses it at higher doses (> 20 nmol; Wildsoet & Pettigrew, 1988b; Ehrlich, Sattayasai, Zappia & Barrington, 1990). Apparently, drugs suppress deprivation myopia at high doses (Fig. 5) because they block the translation of visual experience into axial eye growth unspecifically.

Effects of different drugs on deprivation myopia and lens-induced refractive errors

The major reason for studying the relationship of the retinal dopamine/melatonin system to development of experimental refractive errors was the striking change in growth rhythms with both occluders and lenses (Fig. 2).

Role of dopamine. The way by which dopamine is implicated in the visual control of axial eye growth remains unclear. In those cases where dopamine levels were lowered by drugs (6-OHDA, continuous light, reserpine), suppression of deprivation myopia was observed. On the other hand, if dopamine levels were raised (after sulpiride application) or left unchanged (5,7-DHT, or melatonin in the present study), deprivation myopia was enhanced. However, without testing more drugs, no generally valid rule can be derived. At least, at this stage, predictions regarding the action of other drugs that change retinal dopamine levels could be made and tested. The role of dopamine during development of lens-induced refractive errors is also unclear. The differences in the DOPAC/DA ratio between the two eyes treated with lenses of different sign were small but significance was achieved because the observations were paired. The absolute DOPAC/DA ratios were quite variable among groups (see Table 1).

Role of serotonin. Additional experiments with other drugs are also necessary to uncover a possible role of serotonin in the visual control of eye growth. In our studies, with lowered serotonin levels, deprivation myopia was both suppressed (reserpine, continuous light (Bartmann *et al.*, 1994)) or enhanced (5,7-DHT, sulpiride) or was enhanced while the levels remained unchanged (melatonin).

Is melatonin involved in the development of deprivation myopia? Caution is necessary with regard to an involvement of melatonin in deprivation myopia. It has recently been shown that, even if most serotonergic cells (amacrine cells and bipolar cells) are destroyed in the chicken retina by kainate, circadian rhythms of activity of *N*-acetyl transferase and of melatonin levels in the photoreceptors persist (Zawilska & Iuvone, 1992; Thomas *et al.*, 1993). Therefore, from our measurement of serotonin, nothing can be said about melatonin levels. Hoffmann and Schaeffel (1994), using radioimmunoassays, found that diurnal retinal melatonin rhythms are, in fact, not changed during development of deprivation myopia but seem to be largely uncoupled from diurnal dopamine rhythms; the observed disturbances of the diurnal dopamine rhythms are then apparently generated by sources other than the melatonin rhythms in the photoreceptors. Changes in deprivation myopia as observed in the current study after direct melatonin application would then be quite unexpected. Normally, melatonin has a suppressive effect on dopamine. Nowak *et al.* (1992) found that dopamine synthesis was reduced 1 hr after intravitreal melatonin application. We assume that the lack of a change in dopamine levels at the end of our melatonin experiment is explained by the fact that the half life time of melatonin is very short (approx. 30 min; Stankov, Gervasoni, Scaglione, Perego, Cova, Marabini & Fraschini, 1993). The intravitreal dose of melatonin necessary to change deprivation myopia in our study appears high compared to the half saturation concentration for the chicken melatonin receptors in the inner plexiform layer (around 50 pmol; Laitinen & Saavedra, 1990). On the other hand, previous studies involved comparable doses of intravitreal melatonin until effects on *N*-acteyl transferase activity could be detected (Nowak *et al.*, 1992). Electroretinographic studies on the possible toxicity of the effective doses are underway.

Effects of reserpine on lens-induced growth changes

We found initially that lens-induced growth changes were partially suppressed in reserpine injected eyes. In the subsequent experiments the effects of lenses of different sign were studied more detailed and unexpected results were found: only the effects of negative lenses were suppressed (Fig. 4). Since axial elongation was suppressed by reserpine no matter whether it was produced by deprivation or negative lenses, it could be speculated that reserpine had a direct effect on scleral growth. However, since scleral growth was not entirely suppressed but continued as in eyes with normal vision (Fig. 4), a gradual reduction must be postulated. Negative lens-induced axial elongation is blocked by optic nerve section (Wildsoet & Wallman, 1995) while deprivation-induced axial elongation continues. Reserpine would have to act on both mechanisms in the same fashion. It remains unclear why reserpine blocks selectively the effects of negative lenses but one explanation would be that choroidal thickness changes (as induced by positive lenses) are not affected by reserpine. In a previous study (Schaeffel *et al.*, 1994a) a drug that lowered dopamine levels but did not affect serotonin levels (6-OHDA) had no effect on lens-induced growth changes for lenses of either sign. Since the inhibition of negative lens-induced growth effects is accompanied by a drop in serotonin levels and the disappearance of labelling of serotonergic cells in the retina [Fig. 3(D)], it might be that serotonin is involved in the negative lens-induced axial elongation. The latter hypothesis could be tested by raising chickens with negative lenses after 5,7-DHT application.

Variability of deprivation myopia and retinal dopamine levels

A striking feature of deprivation myopia is its inter-individual variability which results from genetical differences among the animals (Schaeffel & Howland, 1991). The observation is interesting with regard to human refractive errors because the susceptibility to presumably visually-acquired refractive errors (like school myopia) is also quite different. It would be important to identify factors that determine how much myopia develops in response to changed visual experience. One rather simple hypothesis was raised by Li *et al.* (1994) that individual differences in dopamine levels are a factor. We tested this hypothesis in chickens (Fig. 6) but it is clear now that the determinants must be more complex. The results indicate that static levels of dopamine are not critical for the "gains" of the mechanisms producing deprivation myopia.

Common features of both deprivation-triggered and lens-triggered mechanisms and a hypothesis where they might converge

Although there is some evidence for the presence of two different feedback loops for the visual control of axial growth in chicks (Schaeffel & Howland, 1988; Schaeffel *et al.*, 1994a, b; Bartmann *et al.*, 1994; Schmid, Wildsoet & Pettigrew, 1993; Wildsoet & Wallman, 1992), there are also similarities in the growth changes with occluders and lenses: both produce changes in choroidal thickness before scleral growth is affected (Wallman, Xu, Wildsoet, Krebs, Gottlieb, Marran & Nickla, 1992; Irving, Sivak & Callender, 1993), both modulate diurnal growth rhythms of either the sclera or the thickness of the choroid (Weiss & Schaeffel, 1993, and this study) and both result in changes in retinal dopamine metabolism (Fig. 6).

It has been shown that choroidal blood flow is reduced during deprivation (Shih, Malinda, Fitzgerald & Reiner, 1993b). Moreover, it has been shown that electrical stimulation of the Edinger–Westphal nucleus can change choroidal blood flow (Shih *et al.*, 1993b). We have found that chickens change their accommodation tonus in response to lens treatment (Schaeffel *et al.*, 1988) but that growth changes of the eye are not initiated by changes in the ciliary muscle tonus (Schwahn & Schaeffel, 1994). Because the tension of the ciliary muscle has no effect, we propose as a working hypothesis that, during lens treatment, changes in accommodation tonus are mediated to the eye growth mechanisms by a different pathway. Since the activity of the Edinger–Westphal nucleus modulates choroidal blood flow, it could be that the information on the average accommodation is integrated after transmission through the third nerve, the ciliary ganglion and, finally, the choroidal nerves from the ciliary ganglion to the choroid (Shih *et al.*, 1993b). Because the choroid seems to be a target common to both deprivation-triggered and lens-triggered mechanisms, we propose that they both merge at this point (Fig. 7; inputs to the Edinger–Westphal nucleus are greatly simplified).

In summary, there appear three mechanisms operating to visually control axial eye growth in chickens.

Mechanism 1. Local retinal image degradation produces locally choroidal thinning and enhanced scleral growth ("deprivation myopia"). The mechanism does not require a connection to the brain and involves the retinal dopamine system.

Mechanism 2. Imposed positive defocus (produced either by recovery from deprivation myopia or by positive lenses) produces rapid choroidal thickening which moves the retina anteriorly and reduces myopia. The mechanism is not affected by reserpine, 6-OHDA or continuous light. The mechanism appears to be local since optic nerve section does not block it (Wildsoet & Wallman, 1995).

Mechanism 3. Imposed negative defocus (produced by negative lenses) generates axial elongation of the eye. It seems unlikely that mechanism (3) operates locally in the eye since the growth response to negative lenses is reduced or suppressed after optic nerve section (Wildsoet & Wallman, 1995). Like mechanism (1), the mechanism is blocked by reserpine. We propose that it is driven by the accommodation tonus; we have shown that the information on accommodation does not reach the eye via the ciliary nerves (Schwahn & Schaeffel, 1994). A more likely candidate are the choroidal nerves.

Since all mechanisms (1)–(3) affect choroidal thickness, we propose that (1) and (2) have a direct local effect on choroidal metabolism and blood flow and that (3) has access to the choroid via the choroidal nerves. It remains to be explained how "emmetropization" can occur with lenses after lesions of the Edinger–Westphal nucleus: since "mechanism 1" produces axial elongation proportional to the amount of image degradation, a kind of rough emmetropization is achieved by maximizing retinal image contrast (Bartmann & Schaeffel, 1994).

In conclusion, we are only at the beginning of uncovering the complex interactions of "local" and "central" visual control of eye growth in the chicken, and of the neuromodulators and transmitters involved. However, since the animal model and the required techniques are readily available, much progress can be expected in the future.

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