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Dopaminergic agonists that result in ocular growth inhibition also elicit transient increases in choroidal thickness in chicks

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Abstract

The dopaminergic system has been implicated in ocular growth regulation in chicks and monkeys. In both, dopamine D2 agonists inhibit the development of myopia in response to form deprivation, and in chicks, to negative lenses as well. Because there is mounting evidence that the choroidal response to defocus plays a role in ocular growth regulation, we asked whether the effective agonists also elicit transient thickening of the choroid concomitant with the growth inhibition.

Negative lenses mounted on velcro rings were worn on one eye starting at age 8–12 days. Intravitreal injections (20 μ l; dose=10 nmole) of the agonist (dissolved in saline) or saline, were given through the superior temporal sclera using a 30G needle. Eyes were injected daily at noon, for 4 days, and the lenses immediately replaced. Agonists used were apomorphine (non-specific; n=17), quinpirole (D2; n=10), SKF-38393 (D1; n=9), and saline controls (n=22). For the antagonists, the same protocol was used, but on each day, the lenses were removed for 2 hours. Immediately prior to lens-removal, the antagonist was injected (20 μ l; dose=5 nmole). Antagonists used were methylergonovine (non-specific; n=12), spiperone (D2; n=20), SCH-23390 (D1 n=6) and saline controls (n=27). Comparisons to saline (continuous lens wear) controls were from the agonist experiment. Axial dimensions were measured using high frequency A-scan ultrasonography at the start of lens wear, and on day 4 prior to the injections, and then again 3 hours later. Refractive errors were measured using a Hartinger's refractometer at the end of the experiment.

Apomorphine and quinpirole inhibited the refractive response to the hyperopic defocus induced by the negative lenses (drug vs saline controls: -1.3 and 1.2 D vs -5.6 D; $p<0.005$ for both). This effect was axial: both drugs prevented the excessive ocular elongation (change in axial length: 233 and 205 μ m vs 417 μ m; $p<0.01$ for both). Both drugs were also associated with a transient thickening of the choroid over 3 hours (41 and 32 μ m vs -1 μ m; $p<0.01$; $p=0.059$ respectively) that did not summate: choroids thinned significantly over the 4 day period in all lens-wearing eyes.

Two daily hours of unrestricted vision during negative lens wear normally prevents the development of myopia. Spiperone and SCH-23390 inhibited the ameliorating effects of periods of vision on lens-induced refractive error (-2.9 and -2.8 D vs 0.6 D; $p<0.0001$), however, the effects on neither axial length nor choroidal thickness were significant. These data support a role for both D1 and D2 receptors in the ocular growth responses.

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Keywords

Emmetropization; chick; choroid; myopia

Introduction

Dopamine is a neurotransmitter found in a subset of amacrine cells in most vertebrates, and in interplexiform cells in some species (Kramer, 1971; Dowling and Ehinger, 1978; Witkovsky and Dearth, 1992). The functions attributed to dopamine are numerous, predominant among these is as a mediator of light-adaptive changes in retinal circuitry and in RPE physiology, including photoreceptor retinomotor movements, pigment dispersal in RPE cells, and horizontal cell uncoupling. It is also an integral component of the retinal circadian oscillators, functioning as the “day” signal in a mutually-inhibitory reciprocal relationship with the hormone melatonin (reviews: Witkovsky and Dearth, 1992; Witkovsky, 2004). Another potential function that may be unrelated to diurnal phenomena and light adaptive mechanisms is as a signal molecule in the visual regulation of eye growth. In both chickens and monkeys, dopamine content decreases in retinas of fast growing eyes developing axial myopia in response to deprivation of form vision (Stone et al., 1989; Iuvone et al., 1991; Rohrer et al., 1993) or to hyperopic defocus induced by negative lens wear (Guo et al., 1995). Conversely, dopamine content increases in retinas of slow-growing eyes recovering from form deprivation myopia (Pendrak et al., 1997). Furthermore, intravitreal injections of the dopamine agonist apomorphine prevents the development of deprivation-induced myopia in both chicks and monkeys, and negative lens-induced myopia in chickens (Schmid and Wildsoet, 2004). More recent work showed that exogenous dopamine (Gao et al., 2006) and its precursor levodopa (Mao et al., 2010) have similar preventative effects on myopia development induced by form deprivation in rabbits and guinea pigs, respectively. Finally, the amount of myopia induced by varying amounts of image degradation is directly proportional to the reduction in retinal dopamine in chicks (Stone et al., 2006).

The finding that the D2 receptor agonist quinpirole, but not the D1 agonist SKF-38393 was effective in inhibiting form deprivation-induced myopia (McCarthy et al., 2007) argues for a D2 receptor-mediated mechanism. Accordingly, the D2 receptor antagonist sulpiride enhanced deprivation-induced myopia (Schaeffel et al., 1995). By the same token, when the D2 antagonist spiperone was co-administered with apomorphine, it attenuated the protective effect of apomorphine (Rohrer et al., 1993) and when injected prior to daily periods of unrestricted vision in form deprived chick eyes, it prevented the ameliorative effect of the vision on the development of myopia (McCarthy et al., 2007).

The purpose of the present study was to further examine the role of dopamine in the signal cascade mediating emmetropization. Specifically, we asked whether the growth inhibition effected by D2 receptor agonists in negative lens-wearing eyes was consistently associated with increases in choroid thickness, which would be expected if the choroidal response is part of the signal pathway leading to ocular growth inhibition, as has been suggested (Nickla, 2007). If this were true, it would follow that D1 agonists would not affect choroidal thickness. Furthermore, injections of a specific D2 antagonist should counter the growth inhibitory effects of daily periods of vision, and not be associated with choroidal thickening. We found that the effective growth inhibitors apomorphine (non-specific) and quinpirole (D2 agonist) both resulted in a transient choroidal thickening, while the relatively ineffective D1 agonist SKF-38393 did not. Contrary to expectation (McCarthy et al., 2007), we found that the D2 antagonist spiperone had only a partial effect in preventing the refractive inhibition normally induced by periods of vision in lens-wearing eyes; these eyes became less myopic than no-vision saline-injected lens-wearing eyes. The choroidal response was not affected. While these

data are consistent with previous work indicating a role for D2 receptors, they also suggest involvement by the D1 receptor family as well. Some of these results have been presented in abstract form (Dhillon and Nickla, 2008). Part of the data in Figure 1C has been published (Nickla and Wallman, 2010).

Methods

Subjects

Subjects were White Leghorn chickens (*Gallus gallus domesticus*), hatched in an on-site incubator and raised in temperature-controlled brooders. The light cycle was 12L/12D (8:00 am to 8:00 pm). Food and water were supplied ad libitum. In all experiments, the right eye was treated and the left eye served as the untreated controls. Care and use of the animals conformed to the ARVO Resolution for the Care and Use of Animals in Research.

Experimental design

Agonists—Negative lenses (-10 D) mounted on velcro rings were attached to the matching ring that was glued to the feathers around one eye, starting at age 8–12 days. (There are no age-related differences in the responses to negative lens-wear over this range of age (Wildsoet and Wallman, 1995). On each day for 4 days, chicks were anaesthetized with isoflurane inhalation anesthesia, and intravitreal injections (20 μ l, for a dose of 10 nmoles injected) of the drug dissolved in saline, or saline (0.75%; n=22) were given at approximately noon. Each experiment had a number of saline controls to control for inter-experiment variability; these data were combined, as there were no significant differences between experiments. Injections used a 30G needle, going through the skin of the lids over the superior temporal sclera after removing the feathers and cleaning the skin with alcohol. Care was taken to use the same injection site for subsequent injections. The needle remained in place for 30 seconds before being slowly withdrawn while the skin around the site was held tightly together using a small forceps. The lenses were replaced immediately. The agonists used (all Tocris Bioscience) were apomorphine (non-specific; n=17), quinpirole (n=10; D2/D4 selective: Sullivan et al., 1998), SKF-38393 (n=9; K_i for D1 vs D2, D3, D4 receptors =1.0 vs 150, 5000, 1000 nM; Seeman and Van Tol, 1994). The data from this group were also used in the analysis of the effects of the antagonists (Figure 2).

Antagonists—For dopamine antagonists, the same protocol as above was used, however, on each day for 4 days the lenses were removed for a 2-hour period starting at around noon. Immediately prior to lens removal, the following drugs were injected, in 10 μ l, for a dose of 5nmole: spiperone (Tocris; n=20), a D2/D4 receptor antagonist (K_i for D2 vs D3, D4, D1, D5 = 0.06 vs 0.6, 0.08, 350, 3500; Seeman and Van Tol, 1994), SCH-23390 (Sigma; n=6), a D1 receptor antagonist, methylergonovine maleate (Sigma; n=12), a non-specific dopaminergic antagonist, or saline (n=27) as injection controls. In graphs, “sal/vis” refers to the saline-injected group, in which the lenses were removed daily, like the drug-injected groups. The data from saline-injected eyes wearing lenses continually are from the agonist experiments described above, and are denoted “sal/lens” in graphs. For spiperone, the procedure used by Ashby et al. (personal communication; Ashby and Schaeffel, 2010) was followed. Spiperone was dissolved in a 1mg/ml solution of ascorbic acid to yield 500 μ M concentration, and heated to 30 degrees for 10 minutes while stirring. Doses for all drugs were based on the results of McCarthy et al. (2007).

For all experiments, axial dimensions were measured using high frequency A-scan ultrasonography (details in Nickla et al., 1998) at the start of lens wear, and on day 4 immediately prior to the injections, and then again 3 hours later. Refractive errors (RE) were measured using a Hartinger's refractometer (details in Wallman and Adams, 1987) at the end

of the experiment. Statistical analyses between groups used an ANOVA and post-hoc Dunnett adjustment.

Results

Dopamine agonists

We examined the effects of three dopaminergic agonists on the refractive responses to hyperopic defocus induced by negative lens-wear (ANOVA, $p=0.0001$; Figure 1A). Both the non-specific agonist apomorphine and the D2 receptor agonist quinpirole significantly inhibited the development of myopia in response to negative lenses (RE respectively vs saline controls: -1.3 D and 1.2 D vs -5.6 D; $p<0.005$ for both). The D1 receptor agonist SKF-38393 was not effective (-2.8 vs -5.6 , $p=0.15$; note, however, that using a two-sample t-test $p=0.03$, perhaps showing a partial efficacy). The inhibition of myopia development for both apomorphine and quinpirole was the result of inhibiting axial elongation (ANOVA, $p<0.005$; Figure 1B): the drug-injected eyes grew significantly slower than those injected with saline (change in axial length respectively: 233 and 205 vs 417 μm ; $p<0.01$ for both). Consistent with the lack of a refractive effect, SKF-38393 had no effect on axial elongation (395 vs 417 μm). It should be noted here that the lack of a significant difference in axial elongation between the fellow (contralateral) eyes and those of the saline-injected lens-wearing eyes is presumably due to an injection effect that is unavoidable in eyes receiving multiple injections (see discussion of this in Rohrer et al., 1993). The relevant statistical comparison is between the drug-injected eyes and the saline-injected eyes.

Both effective growth inhibitors resulted in increases in choroidal thickness (ANOVA, $p<0.005$; Figure 1C: apomorphine vs saline: 41 vs -1 μm , $p<0.01$; Quinpirole: 32 vs -1 μm ; $p=0.059$). This effect was gone by 24 hours, and did not alter the normal choroidal thinning in response to the negative lenses (change over 4 days: -154 and -123 μm vs -137 μm ; Figure 1D). SKF-38393 had no effect on choroidal thickness (Figure 1C, but note the large variability). In summary, only those drugs that blocked the development of myopia also induced transient increases in choroidal thickness.

Dopamine antagonists

Normally, allowing 2 hours per day of unrestricted vision to eyes wearing negative lenses largely prevents the development of axial myopia. In these experiments we examined the effects of three dopaminergic antagonists for their ability to block the vision-induced inhibition of lens-induced myopia, and their effects on the vision-induced choroidal response. We found that both spiperone and SCH-23390 (but not methylergonovine; data not shown) blocked the vision-induced inhibition in the refractive development of myopia (ANOVA $p<0.0001$) (Figure 2A, drug vs sal/vis: -2.9 and -2.8 D vs 0.6 D respectively; $p<0.005$ for both). However, this effect was not complete: while the myopia induced by the lenses in both groups was significantly greater than in saline-injected vision controls, it was significantly *less* than that of eyes wearing lenses continually (-2.9 and -2.8 D vs -5.6 D; $p<0.005$). This partial refractive effect for spiperone is consistent with its effect on axial elongation (Figure 2B; ANOVA $p=0.01$); eyes tended to grow faster than saline-injected eyes, but the difference did not reach statistical significance (drug vs saline: 338 vs 273 μm ; $p=0.17$). There was no significant effect on the axial elongation of eyes injected with SCH-23390 or methylergonovine (316 and 246 μm vs 273 μm ; data for methylergonovine not shown).

Neither spiperone nor SCH-23390 had a significant inhibitory effect on the vision-induced transient choroidal thickening (Figure 2C; ANOVA $p<0.0001$): choroids of both showed similar thickening as did the saline/vision controls (27 and 48 μm vs 45 μm ; for spiperone, $p=0.17$). Methylergonovine had no effect on the choroidal response (data not shown).

Discussion

There are two groups of dopamine receptors, both of which are coupled to G-proteins. The D2-like family (D2, D3, D4) inhibits adenylate cyclase and cAMP, while the D1-like family (D1 and D5) stimulates adenylate cyclase and cAMP (review: Witkovsky, 2004). D1-like receptors predominate in the retina: they are found on bipolar, horizontal, amacrine and ganglion cells (Veruki and Wassle, 1996) while D2-like receptors are found on RPE cells (Rohrer and Stell, 1995) and as autoreceptors on amacrine cells (Veruki, 1997). As previously discussed, most of the available evidence supports a D2 receptor-mediated role for the dopaminergic effects on the emmetropization system in chicks (Rohrer et al., 1993; McCarthy et al., 2007; Ashby and Schaeffel, 2010), however, early work by Stone and colleagues suggests a more complex picture that includes potential interactions between both the D1 and D2 systems (Stone et al., 1990). Our data support the latter hypothesis.

We find that the ocular growth inhibition produced by intravitreal injections of apomorphine or quinpirole into eyes wearing negative lenses resulted in significant transient increases in choroidal thickness, similar to that found in response to brief daily periods of vision that also inhibit eye growth (Nickla, 2007). The D1 agonist SKF-38393 was not effective in growth inhibition, nor did it elicit choroidal thickening. Unlike McCarthy et al. (2007; and see also Rohrer et al., 1993), we were not able to completely inhibit the effects of daily periods of vision using the D2 antagonist spiperone. In our hands, spiperone had a *partial* efficacy in preventing the vision-induced protection from myopia, as did the D1 antagonist SCH-23390. This partial refractive effect was associated with a partial inhibition of the vision-induced choroidal thickening, although this did not reach significance. One possible explanation for these findings is that spiperone cross-reacts with other receptors: it binds D4 receptors with equal potency (Patel et al., 2003), and also binds serotonin and alpha-adrenergic receptors (Peroutka et al., 1977; Quirk et al., 1978). In accord with this partial effect, Stone et al. (1990) reported that co-administration of either spiperone or SCH-23390 with apomorphine into form deprived chick eyes reduced axial elongation by about half-maximum, reflecting a partial antagonist effect for both drugs on the dopaminergic control of eye growth. It is worth noting here that in our hands, eyes injected with the D1 receptor agonist SKF-38393 were not as myopic as the saline injected lens-wearing eyes (two-sample t-test $p=0.03$), although the difference did not reach significance using the post-hoc Dunnett adjustment. Our data argue for an interaction between both dopamine receptor families in eye growth regulation. The relative greater sensitivity of the D2 versus D1 receptors (nanomolar vs micromolar) might also play a role in the larger effects of D2 agonists, especially given that the two receptor families are differentially distributed in the retina and RPE, with D1 predominating in retina (Veruki and Wassle, 1996) and D2 on the RPE (Schorderet and Nowak, 1990; Rohrer and Stell, 1995).

An alternative explanation that could account for the discrepancies between our study and those of McCarthy et al. (2007), Rohrer et al. (1993) and Ashby and Schaeffel (2010) is that the underlying dopaminergic mechanisms may differ between form deprivation and negative lens-wear, such that D2 mechanisms may predominate when there is no visual feedback from defocus, while both D1 and D2 receptors may be involved in the presence of hyperopic defocus. There is early evidence in support of underlying pharmacological differences between these two visual manipulations. It was originally reported that the neurotoxin 6-hydroxydopamine suppressed the development of myopia in response to form deprivation, but did not alter the response to negative lenses, supporting different mechanisms (Schaeffel et al., 1994). Yet, three years later the same lab showed a suppressive effect of the drug on lens-wear similar to that in the deprivation paradigm if the power of the lenses was increased (Diether and Schaeffel, 1997), weakening this hypothesis. However, there has been further evidence supporting different underlying retinal mechanisms. First, Schmid and Wildsoet (2004) reported that atropine had a greater inhibitory effect than the combination of atropine and apomorphine on

the development of myopia in form deprived eyes, but this was not true in eyes wearing negative lenses. Second, constant light, which reduces retinal dopamine levels, inhibited the development of form deprivation myopia, but had no effect on negative lens-induced myopia (Bartmann et al., 1994). Third, optic nerve section prevented the development of myopia in negative lens wearing eyes, but not in form deprived eyes (Wildsoet and Wallman, 1995). Finally, the growth response to negative lenses occurs much more rapidly than that to form deprivation (Kee et al., 2001). To conclude, it is possible that the results of this study support different underlying dopaminergic mechanisms between form deprivation and negative lens wear.

Dopamine, the choroid and ocular growth inhibition

We find that the ocular growth inhibition effected by the non-specific dopamine agonist apomorphine and the D2 receptor agonist quinpirole is associated with transient increases in choroid thickness, while the relatively ineffective D1 agonist does not elicit the choroidal response. This transient choroidal thickening is similar in time course (i.e. it decays by 24 hours) and in magnitude as the choroidal response to daily brief periods of unrestricted vision given to eyes that were form deprived, wearing negative lenses, or in otherwise constant darkness (Nickla, 2007), supporting a mechanistic link between choroidal thickening and ocular growth inhibition, and also consistent with dopamine being a mediator of the visual stimulus that counters the myopiagenic effects of deprivation, darkness and hyperopic defocus. It follows that if the antagonists had completely blocked the effects of the daily vision, the choroidal response should also have been absent or attenuated. In fact, there was only a partial antagonism of the daily vision by both antagonists, and this was associated with choroidal thickening, suggesting that the amount of thickening is not related in a “dose-dependent” way to the ocular growth inhibition. However, these results could also be interpreted as constituting evidence that the choroidal thickening response and ocular growth inhibition are, in fact, mediated by separate pathways.

If dopamine is involved in the visual regulation of ocular growth, as proposed, what is the underlying mechanism? Dopamine synthesis and release is modulated by light; it is the chemical mediator of light adaptation (review: Witkovsky, 2004). One hypothesis proposes that dopamine mimics the particular effects of vision that are absent in the absence of form vision (as would occur in form deprivation and constant darkness), for instance, spatial or temporal contrast. Consistent with this hypothesis, the development of myopia in form deprived eyes is prevented by exposure to 12 Hz stroboscopic stimulation (Gottlieb and Wallman, 1987)(Kee et al., 2001), this frequency being compatible with effects on retinal neuronal activity. In fact, only 2 daily hours of strobe stimulation is sufficient to inhibit ocular growth; of note, the episodes of strobe produce significant increases in choroidal thickness (Nickla, 2007), linking this response to ocular growth inhibition. Interestingly, flickering light results in an increase in dopamine release (Umino et al., 1991; Weiler et al., 1997), supporting the idea that dopamine is the chemical mediator of the visual stimuli, possibly temporal transients, that inhibit myopia development. Our finding that the effective growth-inhibiting dopamine agonists result in choroidal thickening as well, are in accord with the hypothesis that dopamine is the chemical mediator of visual stimuli which results in choroidal thickening and ocular growth inhibition.

While this is an attractive hypothesis for dopaminergic effects on form deprivation or constant darkness, it is more difficult to reconcile this notion to the inhibition of myopia in response to negative lens wear, because it is unlikely that hyperopic defocus would affect temporal transients. In accord, stroboscopic stimulation has a weaker effect in inhibiting the myopia development in response to negative lenses compared to form deprivation (Kee et al., 2001), and it is associated with a smaller choroidal response as well (Nickla, 2007). However, because

dopamine agonists are effective in blocking the development of myopia in response to negative lens wear (Schmid and Wildsoet, 2004 and present study), dopamine levels are decreased in these retinas (Guo et al., 1995), and the growth inhibition is associated with transient choroidal thickening, it appears likely that similar dopaminergic mechanisms mediate the inhibitory effects of the visual stimuli here. Furthermore, it indicates that dopamine acts upstream of the choroidal response, possibly on the same pathway that results in ocular growth inhibition. If this were true, then the effective antagonists should also prevent the vision-induced choroidal thickening. Our finding that spiperone only partially blocks the effects of vision on the refractive development of myopia can be interpreted in several ways. If the effect on the choroid is also deemed to be partial (27 μm vs 45 μm for the full response; two sample t-test $p=0.07$), then the most parsimonious interpretation is that there was a partial inhibition of ocular growth as well, similar to the finding of smaller choroidal responses associated with smaller axial effects with strobe stimulation (Nickla, 2007). The alternative interpretation is that the choroidal and scleral responses are independent responses to the same visual stimulus, and that dopamine D2/D4 receptors are not directly involved in mediating the choroidal response but are involved in ocular growth inhibition.

To summarize, accumulating evidence supports a role for dopamine in the signal cascade mediating emmetropization, however, the elucidation of its source and target cells requires further study. Both D1 and D2 receptors are found on retinal neurons, including photoreceptors, and D2 receptors are on both apical and basal membranes of the RPE. If dopamine acts upstream of the choroidal response, as supported by our data, the finding that dopamine stimulates the release of retinal nitric oxide (NO) in response to flickering light (Sekaran et al., 2005) is interesting because NO may be involved in mediating choroidal thickening: Inhibiting NO using nitric oxide synthase inhibitors prevents the choroidal thickening response to myopic defocus, and disinhibits ocular growth (Nickla and Wildsoet, 2004) (Nickla et al., 2009). We speculate that the release of dopamine from the retina in response to visual stimuli triggers the release of NO from either the retina or choroid, leading to choroidal thickening and ocular growth inhibition. Other evidence suggests an interaction of dopamine with acetylcholine: co-administration of atropine and apomorphine to form deprived chick eyes resulted in a smaller growth inhibition than either drug alone, suggesting that muscarinic antagonists and dopamine agonists work on the same signal pathway and have mutually-antagonistic effects at the retinal level (Schmid and Wildsoet, 2004). Finally, if dopamine mediates the protective effects of light against myopia development, as suggested by Ashby and Schaeffel (2010), this might support a role for dopamine in the preventative effects of outdoor activity in children as well (Rose et al., 2008). In conclusion, we present evidence supporting a role for both D1 and D2 dopaminergic receptors in emmetropization. We speculate that the source of dopamine is retinal, and that it acts upstream of the choroidal thickening in response to growth-inhibiting visual stimuli.

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Figure 1.

Effect of three dopamine agonists on the ocular responses to negative lenses, and saline controls. "Fellow" is data for contralateral eyes in all graphs. "Saline" refers to saline injected lens-wearing eyes. A. Effect on refractive error (ANOVA, $p=0.0001$). Both apomorphine ("apomor"; non specific) and quinpirole ("quinpir", D2) inhibited the development of refractive myopia ($p<0.005$ for both comparisons with saline). SKF-38393 (SKF, D1) had no effect. B. Effect on axial length (ANOVA, $p<0.005$). Both apomorphine and quinpirole inhibited the excessive axial elongation in response to the negative lenses ($p<0.01$ for both). SKF had no effect. C. Effect on choroidal thickness (ANOVA, $p<0.005$). Both apomorphine and quinpirole increased choroid thickness over the 3 hours after the injection ($p<0.01$; $p=0.05$).

respectively). SKF had no effect. Part of this graph was reproduced with permission from: Nickla & Wallman, 2010 © Elsevier. D. Change in choroidal thickness over the 4 days of the experiment. Choroids of all lens-wearing eyes showed significant thinning, as expected. Error bars are standard errors of the mean in all graphs. Asterisks denote significant differences with saline controls in all graphs.

Figure 2.

Effect of two dopamine antagonists on the daily 2 hours of vision-evoked ocular responses in eye wearing negative lenses. “Sal/vis” refers to saline-injected eyes that received the same 2 hours of daily vision as the drug groups; statistical comparisons are between drug groups and this group. “Sal/lens” refers to saline-injected eyes wearing lenses continually; these are the same data as are in Figure 1 (“saline”). A. Effect on refractive error (ANOVA, $p < 0.0001$). Spiperone (“spiper”) and SCH-23390 (“SCH”) inhibited the vision-induced protection from myopia development ($p < 0.005$). B. Effect on axial length (ANOVA, $p = 0.01$). While spiperone tended to result in an increase in ocular elongation compared to saline controls, the effect was not significant. C. Effect on choroidal thickness (ANOVA, $p < 0.0001$). Neither antagonist showed a significant difference from the saline-injected controls.