Dopamine Receptor Subtypes Mediate Opposing Effects on Form Deprivation Myopia in Pigmented Guinea Pigs

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Submitted: January 27, 2017
Accepted: July 28, 2018

Citation: Zhang S, Yang J, Reinach PS, et al. Dopamine receptor subtypes mediate opposing effects on form deprivation myopia in pigmented guinea pigs. *Invest Ophthalmol Vis Sci*. 2018;59:4441–4448. https://doi.org/10.1167/iovs.17-21574

Purpose. We reported previously that changes in dopamine receptor (DR) subtype activation modulate spontaneous myopia progression in albino guinea pigs. To determine if DR control of refractive error development is different than in its normal counterpart, we evaluated the contribution of dopaminergic pathways to emmetropization and form deprivation myopia (FDM) progression in pigmented guinea pigs.

Methods. Monocular myopia was induced by unilateral form-deprivation (FD). The effects of agonists of D1R (SKF38393) and D2R (quinpirole), the corresponding antagonists (SCH23390 and sulpiride), and vehicle were tested by peribulbar injection around FD or untreated control eyes. High-performance liquid chromatography with electrochemical detection quantified retinal and vitreous dopamine (DA) and 4-dihydroxyphenylacetic acid (DOPAC) levels. Ocular refraction and axial dimensions were measured using eccentric infrared photoretinoscopy (EIR) and A-scan ultrasonography, respectively, initially and after 2 or 4 weeks of treatment.

Results. After treatment with any of these four agents for 2 weeks, retinal and vitreal DA and DOPAC levels were not significantly different in drug- and vehicle-treated eyes. Neither agonism nor antagonism of D1R or D2R activity affected emmetropization. In contrast, D1R activation by SKF38393 inhibited FDM progression, while D2R activation by quinpirole augmented this response. On the other hand, D2R antagonism with sulpiride slowed FDM progression while D1R antagonism with SCH23390 had no effect.

Conclusions. In pigmented guinea pigs, D1R activation inhibited, whereas D2R activation enhanced, FDM. These results closely mirror previous findings in albino animals and offer further evidence that DA and its cognate receptors affect refractive error regulation in guinea pigs.

Keywords: dopamine receptor, form deprivation myopia, pigmented guinea pig

Myopia is a refractive error in which, without accommodation, images of distant objects are focused in front of the photoreceptors. There is ample evidence that both genetic and environmental factors contribute to myopia onset.[1] Significant advances have been made in optical methods that correct refractive error and even, in some cases, slow myopia progression. In contrast, although atropine in sufficiently high doses is more effective than any of the current optical treatments, it has many side effects because of the wide spread distribution of muscarinic receptors in the eye and elsewhere. To date there is also no general agreement on the site and mechanism of its antimyopia action. Therefore, there is great interest in identifying novel drug targets that can specifically attenuate or halt myopia progression while minimizing unwanted side-effects.[2]

There is a substantial body of evidence that form deprivation myopia (FDM) is associated with decreases in retinal dopamine (DA) levels, in monkeys,[3] chickens,[4] and guinea pigs.[5] DA involvement in myopia is further supported by the findings that DA supplementation inhibited FDM in rabbits.[6] Similarly, supplementation with L-DOPA, a DA precursor, inhibited FDM in pigmented guinea pigs[7] and spontaneous myopia in their albino counterpart.[8] In addition, the nonselective dopamine receptor (DR) agonist apomorphine (APO) inhibited myopia in chickens,[4] guinea pigs,[5] and rhesus monkeys.[9] Despite DA-level invariance during FD in mice,[10] DR involvement remains plausible, because APO inhibited myopia in this species.[11]

DA exerts its effects through interacting with DRs, which are grouped into two families according to their pharmacological profile and prevailing second messenger coupling. The D1-like family (D1Rs), including D_1 and D_5, mediate responses through activating adenylate cyclase. In contrast, the D2-like family (D2Rs), including D_2, D_3, and D_4, inhibit adenylate cyclase.[12] The retina expresses all DRs except the D_3 receptor subtype.[13] Some reports implicate D2Rs in the inhibitory effect of APO on FDM, while others implicate D1Rs in this process (mechanisms may be species-specific; for reviews see Refs. 2 and 14). In albino guinea pigs, which spontaneously develop myopia in a normal visual environment,[15,16] a D1R agonist (SKF38393) and a D2R antagonist (sulpiride) inhibited FDM, whereas a D2R agonist (quinpirole) and a D1R antagonist (SCH23390) promoted it.[17] Additionally, APO had biphasic effects, promoting spontaneous myopia progression at low doses, but inhibiting it at higher doses.[17] These opposing

Table. Drug Treatment Design

Treatment (Dose, ng)	Sample Size
Normal visual environment	
SKF38393 (0, 10, 100, 1000)	14, 16, 16, 16
SCH23390 (0, 2500)	15, 16
Quinpirole (0, 100, 1000)	31, 22, 30
Sulpiride (0, 2500, 25,000)	13, 18, 16
Monocular FD	
FD+SKF38393 (0, 10, 100, 1000)	13, 13, 15, 14
FD+SCH23390 (0, 250, 2500, 8000)	13, 12, 16, 13
FD+Quinpirole (0, 10, 32, 100)	22, 20, 21, 24
FD+Sulpiride (0, 2500, 25,000)	12, 15, 12

effects suggested that D1R activation suppresses spontaneous myopia progression, whereas D2R activation promotes it, in albino guinea pigs. However, it was unclear whether such effects are unique to albinos, which have structural and functional defects, related to the absence of melanin pigment, that may alter the roles of DRs in regulating this processes.[18] Because of the melanin deficiency in albinos, illumination may damage the retinal pigment epithelium (RPE) and the neural retina.[19] Wang et al.[15] found that albinos have a thinner retina and narrower spaces between outer segments of photoreceptors than their pigmented counterparts. Furthermore, the electroretinograms (ERGs) are different in albino and pigmented guinea pigs.[20] Such structural and functional deficits in the retina and RPE may be relevant to the onset of spontaneous myopia. Thus, the current follow-up study was undertaken to clarify whether the roles of D1R and D2R in ocular growth and myopia, previously identified in albino guinea pigs, apply also in form deprived pigmented guinea pigs, which are a more commonly used myopia model.

We describe here the individual roles of D1Rs and D2Rs in eye growth regulation in pigmented guinea pigs, some of which were form deprived to induce myopia. The results indicate that the D1R and D2R balanced interaction model developed in albino guinea pigs applies not only to albinos, but is also relevant in the control of myopia development in pigmented animals.

Materials and Methods

Animals

The animal usage protocol was approved by the Animal Care and Ethics Committee at Wenzhou Medical University, Wenzhou, China, according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Two-week-old pigmented guinea pigs (*Cavia porcellus*, English short-hair stock, tricolor strain, $n = 625$) were obtained from the animal laboratory center at Zhejiang University in Hangzhou, China. The animals were assigned to different groups to characterize: (1) retinal and vitreous DA and 4-dihydroxyphenylacetic acid (DOPAC) levels ($n = 31$); and (2) refractive error development ($n = 594$; Table). The animals were reared under a daily 12-hour light/12-hour dark cycle (incandescent lights on at 8:00 AM) in the animal facilities, with room temperature maintained at 25°C. Illuminance at the cage floor was approximately 300 lux. The animals had free access to standard food and water and were provided with fresh vegetables twice a day.

Establishment of FD

Monocular FD was induced with a facemask, which covered the right eye while leaving the left eye, nose, mouth, and ears uncovered.[21] The facemask was examined for its integrity and correct placement thrice weekly; if necessary, it was replaced with a new one of suitable size. The facemask was only temporarily removed during drug injection under dim red light.

Pharmacological Manipulation

SKF38393 (D1R agonist), SCH23390 (D1R antagonist), quinpirole (D2R agonist), and sulpiride (D2R antagonist) were obtained from Tocris (Glasgow, UK). SKF38393, SCH23390 and quinpirole were each dissolved in Milli-Q water containing 0.1% ascorbic acid (Sigma-Aldrich Corp., St. Louis, MO, USA) as an antioxidant. Sulpiride was dissolved in dimethylsulfoxide (DMSO; Sigma-Aldrich Corp.) and then diluted with Milli-Q water to obtain the final working concentration (Table) in 0.1% DMSO. After anesthesia of the right eye with a drop of 0.5% proparacaine hydrochloride into the lower conjunctival sac, each drug (0.1 mL) was injected with a 26-gauge needle (0.45 × 16 mm), connected to a 1-mL syringe cannula (Shanghai Kindly Medical Devices Co., Ltd, Shanghai, China), into the inferior peribulbar space. Animals in vehicle control (VC) groups were injected with the respective solvent. All injections were conducted under dim red light, once daily (1 hour after lights on, i.e., at 9:00 AM). The left (fellow) eyes of all animals remained untreated throughout the experiment. Under the normal visual environment (no FD), the treatment period for all four drugs was 4 weeks. In animals experiencing FD, the facemask was taken off a few seconds before the injection and was replaced immediately afterward. The duration of drug treatment of animals with monocular FD was set at 2 weeks for SKF38393 and quinpirole, and 4 weeks for SCH23390 and sulpiride.

Each vehicle (0 ng) or drug-treated group was paired with a subset of age-matched control animals that did not undergo any experimental procedure. The number of such animals exposed to normal vision in the SKF38393-, SCH23390-, quinpirole-, and sulpiride-treated groups was 15, 17, 31, and 14, respectively. For the FD group, the numbers were 13, 13, 21, and 12, corresponding to the above four drug groups. A total of 594 guinea pigs was used to investigate the effects of these DA analogs on refractive error development.

Quantification of DA and DOPAC in Retina and Vitreous

Retinal and vitreous DA and DOPAC levels were quantified using high-performance liquid chromatography with electrochemical detection (HPLC-ECD)[10,22] after a week of daily peribulbar 100-μL injections of either the vehicle or drug. SKF38393, SCH23390, and sulpiride were used at 1, 2.5, and 25 μg, respectively, whereas the quinpirole dose was 100 ng. These doses were selected because each of these drugs at the indicated doses had a significant effect on spontaneous myopia progression in albino guinea pigs.[23] All samples for HPLC analysis were harvested under incandescent room light (illuminance = 300 lux) at 9:00 AM (1 hour after room-light on). The animals were anaesthetized and decapitated to sacrifice them. The eyes were removed and hemisected through the equator, the vitreous was saved, and the retina was carefully dissected away from the choroid. The wet weight of each retina and the volume of each vitreous fluid sample was recorded before freezing. Each frozen sample was homogenized in 100 μL (for the retina) or 15 μL (for the vitreous body) of ice-cold 0.1 M perchloric acid, containing 10 μM ascorbic acid, 0.1 mM ethylenediaminetetraacetic acid (EDTA) disodium salt, and 0.02 μM 3,4-dihydroxybenzyl-amine (as an internal standard). DA and DOPAC levels were assessed

with the Agilent 1200 series neurotransmitter analyzer (Agilent Technologies, Santa Clara, CA, USA), consisting of a G1367B autosampler, G1312A binary pump, G1322A degasser, ANTEC DECRARD SDC electrochemical detector (Antec, Zoeterwoude, The Netherlands) equipped with a Sencell with a 2-mm glassy carbon working electrode, and Acclaim C18 column (2.2 μm, 2.1× 100 mm; Thermo Fisher Scientific, Waltham, MA, USA). Separations were performed at a flow rate of 0.2 mL/min using a mobile phase of phosphate-buffered saline (PBS), containing 0.05 mM EDTA, 1.7 mM orthosilicic acid, 90 mM Na_2HPO_4, 50 mM citric acid, and 5% acetonitrile with the detection cells set at +700 mV. The columns and detector cells were kept at 35°C in a column oven. The data were collected and analyzed by ChemStation (Agilent Technologies).

Ocular Refraction and Biometric Measurements

Refractive errors in the vertical meridian were measured without cycloplegia using an eccentric infrared photorefractor on awake animals.[24] Illuminance of the examination room was dimmed to approximately 5 lux. Three readings were recorded, and the mean was used for statistical analysis.

Vitreous chamber depth (VCD, from the posterior lens surface to the retinal nerve fiber layer) and axial length (AL, from the anterior corneal surface to the retinal nerve fiber layer) were measured in unanesthetized animals by A-scan ultrasonography (AVISO Echograph Class I-Type Bat; Quantel Medical, Clermont-Ferrand, France), with an 11-MHz transducer, on the same day following refraction measurements.[25] Velocities of sound were assumed to be 1534 m/s for the aqueous and vitreous humor, and 1774 m/s for the lens. The cornea was anesthetized with a drop of 0.5% proparacaine hydrochloride (Alcon, Purrs, Belgium). A stand-off rubber tube was attached to the probe tip to avoid indentation of the cornea.[16,26] At least eight traces were captured, and the mean values for VCD and AL were used in statistical analysis.

All biometric parameters were measured immediately before treatment and after 1, 2, and 4 weeks of treatment. In animals undergoing FD, the facemask was taken off 1 minute before a measurement and replaced immediately afterwards.

Statistical Analysis

All data are expressed as the mean ± standard error of the mean (SEM). We confirmed that the data distributions were normal and variance was equal, so that the following parametric tests could be used. Each pair of right and left eye parameter values was compared using paired Student's *t*-test. To identify treatment effects among the different groups, interocular differences (parameter of the treated eye minus that of the untreated fellow eye) were calculated for refractive status and axial components. Two-way repeated measures ANOVAs, followed by post hoc analysis with Bonferroni correction, were applied to assess the effects of drug treatment and treatment duration, by comparing the refractive errors and axial ocular dimensions of vehicle groups with those of drug-treated groups at given times. One-way ANOVA with Dunnett's test compared DA and DOPAC levels as well as DOPAC/DA ratios in drug and vehicle-injected eyes. All statistical analyses were performed using SPSS (Version 16.0). *P* values less than 0.05 were considered to be significant.

Results

In 2-week-old pigmented guinea pigs without FD, used as normal control (NC) for the SKF38393 injection group, the mean refractive error of the right eyes was 6.03 ± 0.27 diopters (D). The corresponding mean VCD and AL were 3.20 ± 0.02 mm and 7.94 ± 0.02 mm, respectively. Mean refractive error, VCD, and AL of the left eyes were 6.13 ± 0.25 D, 3.18 ± 0.02 mm, and 7.92 ± 0.02 mm, respectively. There was no significant interocular difference in the baseline refraction and axial component values of individual animals ($P > 0.05$, paired *t*-test). The refraction and axial dimensions were also similar in the right and left eyes in the NC group in 2-, 3-, 4-, and 6-week-old animals. The mean differences in refractive error between the left and right eyes were 0.1 ± 0.17, 0.52 ± 0.17, 0.33 ± 0.19, and 0.37 ± 0.13 D. The refractive error development in other NC groups were similar (Supplementary Tables S1–S4).

Endogenous DA Release or Turnover Unchanged by Drug Treatment

Vitreal DOPAC content is an indicator of retinal DA release, while the calculated vitreal or retinal DOPAC/DA ratio is a measure of DA turnover in the corresponding tissue. After 2 weeks of daily vehicle injection, in vehicle-treated FD eyes, the DA and DOPAC contents were 0.12 ± 0.01 and 0.13 ± 0.01 ng/mg, respectively, in the retina, and 0.007 ± 0.001 and 0.07 ± 0.003 ng/μL, respectively, in the vitreous; whereas in the non-FD fellow eyes, the contents were 0.16 ± 0.01 and 0.18 ± 0.01 ng/mg, respectively, in the retina, and 0.01 ± 0.0006 and 0.08 ± 0.003 ng/μL, respectively, in the vitreous (Fig. 1). There was a significant decrease in both the retinal and vitreous DA and DOPAC levels in the vehicle-treated eyes undergoing FD for 1 week, compared with levels in the untreated fellow eyes (paired *t*-test, $P < 0.05$). However, there were no significant differences in DA and DOPAC levels in either the retina or the vitreous of FD eyes treated with any of the four agents, compared with those in the vehicle-treated group (see data in Supplementary Tables S1, S2; 1-way ANOVA with Dunnett's test, $P > 0.05$, $n = 10$–13/group). This invariance indicates that none of the drug treatments changed the DA and DOPAC levels in the FD retinas (Figs. 1A, 1B) or the vitreous (Figs. 1D, 1E). The retinal DOPAC/DA ratios were 1.07 ± 0.07, 1.19 ± 0.08, 0.96 ± 0.07, 1.00 ± 0.06, and 1.06 ± 0.05, in the vehicle-, SKF38393-, SCH23390-, quinpirole-, and sulpiride-treated FD eyes, respectively, compared to 1.18 ± 0.07, 1.19 ± 0.09, 1.00 ± 0.07, 1.04 ± 0.06, and 1.11 ± 0.05, in the corresponding untreated fellow eyes. The vitreous DOPAC/DA ratios were 10.62 ± 1.88, 7.63 ± 0.96, 8.23 ± 0.62, 9.72 ± 0.97, and 7.80 ± 0.66, in the vehicle-, SKF38393-, SCH23390-, quinpirole-, and sulpiride-treated FD eyes, respectively, compared to 7.73 ± 0.38, 8.72 ± 0.73, 7.42 ± 0.53, 8.28 ± 0.49, and 7.36 ± 0.47, in the corresponding untreated fellow eyes. Comparison of the FD eyes with fellow eyes in each group revealed that the DOPAC/DA ratios were also unchanged in the retina (Fig. 1C) and vitreous (Fig. 1F) after FD (paired *t*-test, $P > 0.05$, $n = 10$–13/group). Furthermore, the DOPAC/DA ratios were unchanged by each of the four drugs, and not different from those in the corresponding vehicle-treated eyes (1-way ANOVA with Dunnett's test, $P > 0.05$, $n = 10$–13/group). In summary, while FD decreased DA and DOPAC levels in the retina and vitreous, neither the agonists nor antagonists of D1R or D2R, altered any of these FD effects.

Insensitivity of Normal Visual Development to DR Drugs

We determined whether DR modulation affects emmetropization in pigmented guinea pigs in a normal visual environment. The refractive errors, VCDs, and ALs were 4.75 ± 0.33 D, 3.24 ± 0.02 mm, and 8.46 ± 0.03 mm, respectively, in the vehicle-treated eyes, and 5.60 ± 0.32 D, 3.22 ± 0.02 mm, and 8.43 ± 0.03 mm, respectively, in the untreated eyes, after 4 weeks of treatment.

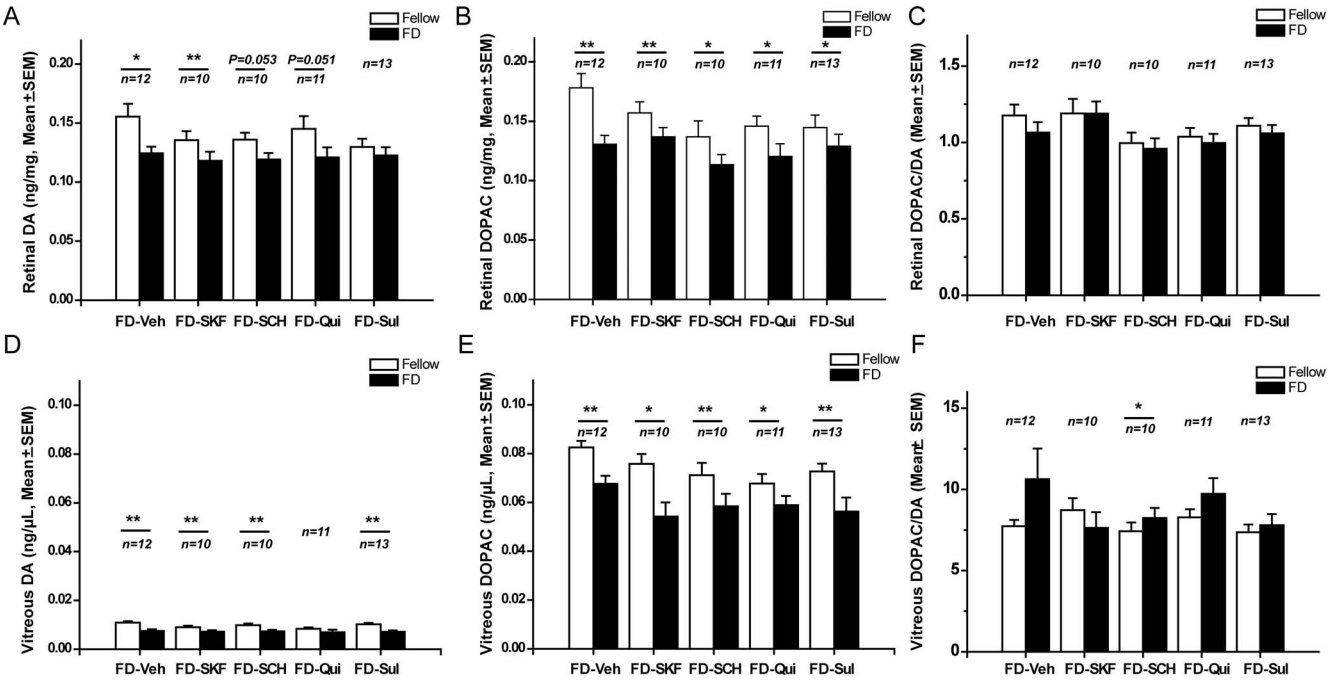

Figure 1. Effects of DR drugs on DA and DOPAC levels. FD decreased concentrations of DA and DOPAC in the retina and vitreous, but DR drugs did not alter DA turnover, as indicated by DOPAC/DA ratios in the retina and vitreous. (**A**) Retinal DA. (**B**) Retinal DOPAC. (**C**) Retinal DOPAC/DA ratio. (**D**) Vitreal DA. (**E**) Vitreal DOPAC. (**F**) Vitreal DOPAC/DA ratio. *Open bar*, fellow eye; *solid bar*, FD eye. SKF38393 (1 µg, FD-SKF, $n = 10$), SCH2390 (2500 ng, FD-SCH, $n = 10$), quinpirole (100 ng, FD-Qui, $n = 11$), and sulpiride (25 µg, FD-Sul, $n = 13$) injected (0.1 mL) into the inferior peribulbar space around FD eyes. One-way ANOVAs with Dunnett's post hoc test evaluated drug effects on DA, DOPAC concentration, and DOPAC/DA ratio, comparing drug- and vehicle-treated groups (all $P > 0.05$). Paired *t*-tests determined whether any of the differences in DA or DOPAC levels between treated and fellow eyes were significant ($^*P < 0.05$; $^{**}P < 0.01$).

After 4 weeks of daily treatment with 1000 ng SKF38393, these values were 4.20 ± 0.46 D, 3.24 ± 0.02 mm, and 8.34 ± 0.02 mm, respectively, in the drug treated eyes, and 5.97 ± 0.31 D, 3.21 ± 0.02 mm, and 8.30 ± 0.02 mm, respectively, in the untreated left eyes. There were no significant differences in refractive errors and axial dimensions between SKF38393-treated eyes and untreated fellow eyes (Supplementary Table S3). Meanwhile, interocular differences in refractive errors, VCDs and ALs for vehicle- and SKF38393 (D1R agonist)-injected groups were all not significantly different, according to 2-way repeated measures ANOVA (refractive error: $F_{3,58} = 0.58$, $P > 0.05$; VCD: $F_{3,58} = 1.31$, $P > 0.05$; AL: $F_{3,58} = 0.56$, $P > 0.05$). Similarly, none of the other drug treatments significantly altered interocular differences relative to those in the vehicle-treated group (see details in Supplementary Tables S3–S6). In summary, neither activation nor antagonism of DR subtypes affected emmetropization in the otherwise untreated eyes.

Sensitivity of Myopia Progression to DR Drugs

SKF38393, delivered into the peribulbar space around form deprived eyes, inhibited FDM in a dose-dependent manner (Figs. 2A–C; Supplementary Table S7), causing declines in refractive error ($F_{3,51} = 12.555$, $P < 0.001$), VCD ($F_{3,51} = 6.812$, $P < 0.01$), and AL ($F_{3,51} = 5.962$, $P < 0.01$), which were statistically significant by 2-way repeated measures ANOVA. The form deprived eyes receiving daily vehicle treatment showed a myopic shift from –0.40 ± 0.21 D to –4.85 ± 0.51 D after 1 week, and to –7.44 ± 0.61 D after 2 weeks. On the other hand, daily treatment of FD eyes for 2 weeks with 10 ng SKF38393 induced a refractive error of –7.19 ± 0.43 D, while treatments with 100 and 1000 ng decreased the errors to –5.18 ± 0.58 D and –4.54 ± 0.51 D, respectively. Such declines in FDM are consistent with suppression of VCD and AL elongation. In vehicle-treated animals, the interocular difference was 0.155 ± 0.02 mm in VCD and 0.158 ± 0.02 mm in AL after 2 weeks. In SKF38393-treated animals, the VCD values were 0.156 ± 0.02, 0.101 ± 0.01, and 0.100 ± 0.01 mm, respectively, and the corresponding AL values were 0.158 ± 0.01, 0.114 ± 0.02, and 0.106 ± 0.02 mm, respectively, after drug treatment with 10, 100, and 1000 ng per day for 2 weeks. Taking into account the differences in treatment duration, Figure 2A shows that, compared with vehicle, 100 and 1000 ng SKF38393 had similar inhibitory effects on myopia after 1 and 2 weeks of FD in refractive error, VCD, and AL (all $P < 0.05$ after Bonferroni correction), whereas 10 ng SKF38393 was ineffective.

Quinpirole treatment promoted FDM progression in a dose-dependent manner (Figs. 2D–F, Supplementary Table S8) causing significant increases in refractive error ($F_{3,83} = 9.940$, $P < 0.001$), VCD ($F_{3,83} = 3.819$, $P < 0.05$), and AL ($F_{3,83} = 4.706$, $P < 0.01$), as revealed by 2-way repeated measures ANOVA. After daily treatment with vehicle (control group), FD induced a myopic shift in refraction from –0.41 ± 0.15 D to –3.96 ± 0.33 D after 1 week, and to –6.86 ± 0.51 D after 2 weeks. Treatment with 32 and 100 ng of quinpirole for 2 weeks significantly increased refractive error to –8.87 ± 0.57 D ($P < 0.01$) and –10.05 ± 0.62 D ($P < 0.01$), respectively; it also significantly changed VCD and AL interocular differences after 2 weeks: VCD by 0.195 ± 0.016 and 0.225 ± 0.013 mm, and AL by 0.193 ± 0.014 and 0.239 ± 0.015 mm, respectively. With a shorter treatment duration of only 1 week, 100 ng quinpirole had myopia-promoting effects on refraction and AL (all $P < 0.05$), and 1000 ng affected all ocular parameters, compared with the vehicle.

To confirm the inhibitory effect of D1R activation on FDM, we tested whether daily treatment with the D1R antagonist SCH23390, at doses of 250, 2500, and 8000 ng, for 4 weeks, instead promoted this process (Figs. 3A–C; Supplementary Table S9). Overall, SCH23390 had no effect on myopia progression, although 2-way repeated measures ANOVA

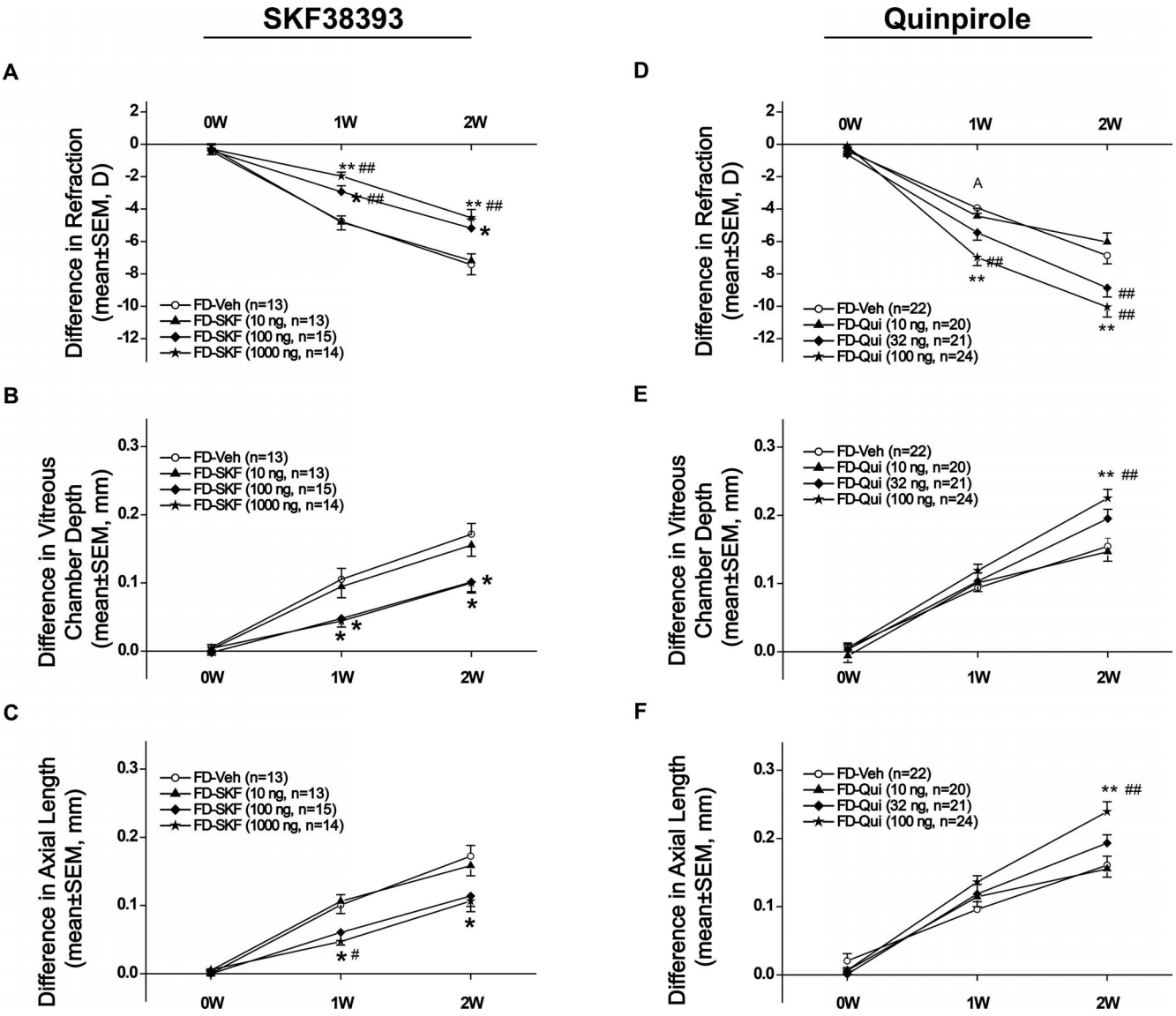

FIGURE 2. D1R and D2R agonists modulate refraction, VCD, and AL. SKF38393 inhibited, while quinpirole promoted, FDM progression. SKF38393 induced changes in the interocular differences in (**A**) refraction, (**B**) VCD, and (**C**) AL. FD-Veh, FD eyes treated with SKF38393 vehicle; FD-SKF, FD eyes treated with SKF38393. $^{*}P < 0.05$ compared to FD-Veh; $^{**}P < 0.01$ compared to FD-Veh; $\#P < 0.05$, $\#\#P < 0.01$, compared to the lowest-dose group (FD-SKF, 10 ng). Quinpirole induced changes in the interocular differences in (**D**) refraction, (**E**) VCD, and (**F**) AL. FD-Veh, FD eyes treated with quinpirole vehicle. FD-Qui, FD animals treated with quinpirole. $^{*}P < 0.05$ compared to FD-Veh; $^{**}P < 0.01$ compared to FD-Veh; $\#P < 0.05$, $\#\#P < 0.01$, compared to lowest-dose group (FD-Qui, 10 ng).

revealed significant differences between drug and vehicle-control treatment in refraction ($F_{3,50} = 4.767$, $P < 0.01$), VCD ($F_{3,50} = 6.318$, $P < 0.01$), and AL ($F_{3,50} = 7.149$, $P < 0.01$). Daily treatment with SCH23390 at each of three given doses had no significant effects on interocular differences during 4 weeks of treatment, compared with VC (all $P > 0.05$).

To confirm the promoting effect of D2R activation on FDM, we determined whether daily treatment with the D2R antagonist, sulpiride, had an opposing effect on this process (Figs. 3D–F; Supplementary Table S10). As predicted, sulpiride treatment inhibited FDM dose-dependently after 4 weeks by reducing induced changes in refractive error ($F_{2,\ 36} = 3.453$, $P<0.05$) and AL elongation ($F_{2,36} = 5.645$, $P < 0.01$), although VCD was invariant ($F_{2,\ 36} = 1.748$, $P > 0.05$), as revealed by 2-way repeated measures ANOVA. After 4 weeks of daily vehicle injection, FD induced a refractive error of –9.20 ± 0.66 D, with corresponding VCD and AL differences of 0.199 ± 0.019 and 0.196 ± 0.023 mm. On the other hand, FD combined with 2.5 and 25 μg sulpiride treatment for 4 weeks induced refractive errors of –8.14 ± 0.72 and –5.54 ± 0.97 D, with corresponding VCD of 0.178 ± 0.025 and 0.138 ± 0.034 mm, and AL of 0.153 ± 0.023 and 0.083 ± 0.032, respectively. Taking treatment duration into account, 2.5 μg sulpiride produced a significant difference in AL elongation only while 25 μg produced significant differences in both refractive error and AL elongation, after 4 weeks of treatment (all $P < 0.05$ after Bonferroni correction).

In summary, neither stimulating nor antagonizing D1R or D2R activity altered refractive development during unobstructed vision (no FD). However, during FD, increasing D1R activity inhibited, whereas increasing D2R activity promoted, FDM progression. Furthermore, D2R antagonism with sulpiride slowed FDM progression, whereas D1R antagonism with SCH23390 had no effect.

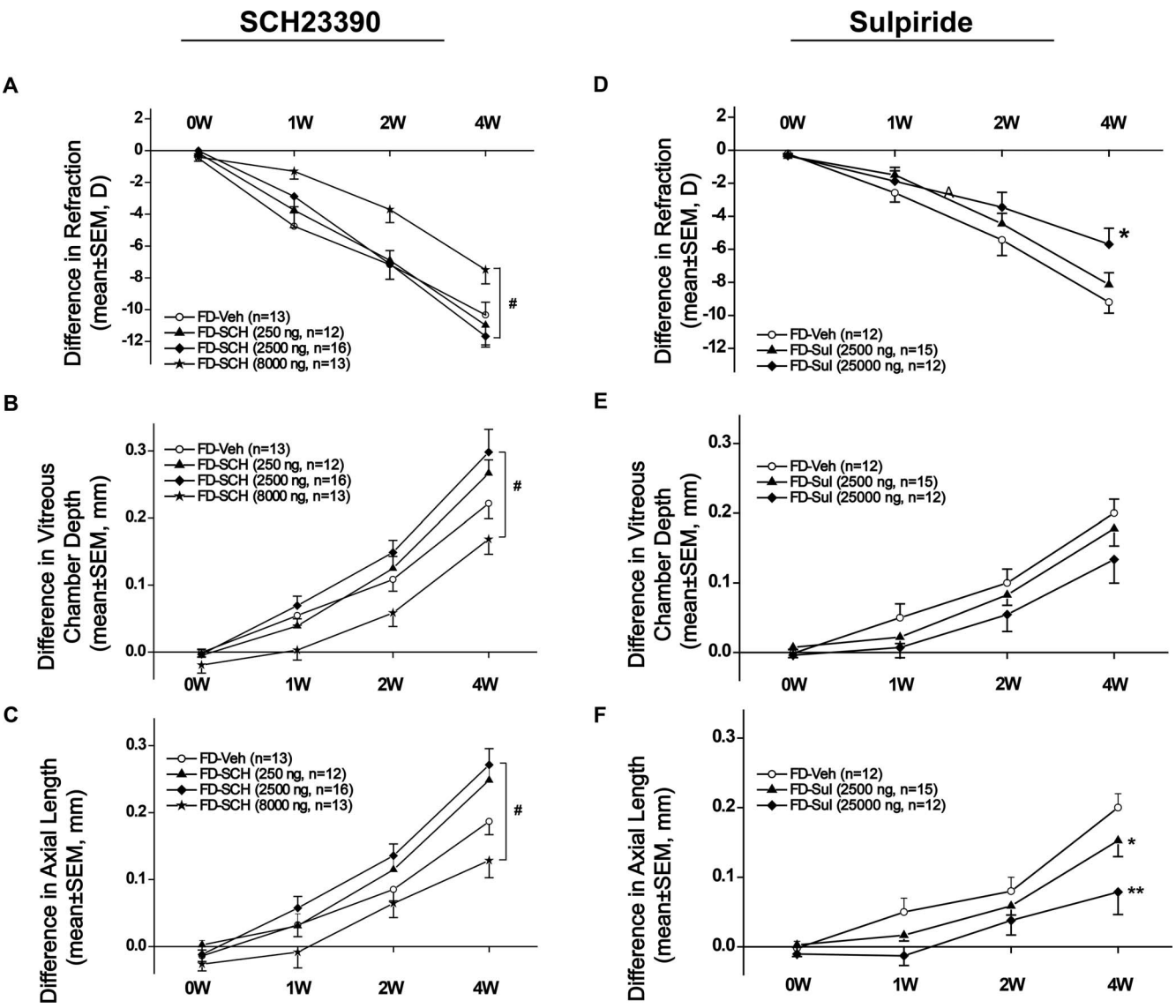

FIGURE 3. D1R and D2R antagonists modulate refraction, VCD, and AL. SCH23390 treatment had no effects on the interocular differences in refraction (**A**), VCD (**B**), and AL (**C**) of FD eyes compared with vehicle-treated eyes, at each time point. FD-Veh, SCH23390 VC undergoing FD. FD-SCH, FD animals treated with SCH23390. #$P < 0.05$ FD-SCH (2500 ng) compared to SCH (8000 ng). Sulpiride treatment inhibited FDM progression, as revealed by the declines in interocular differences in refraction (**D**), VCD (**E**), and AL (**F**) during FDM progression. FD-Veh, sulpiride VC undergoing FD. FD-Sul, FD animals treated with sulpiride. *$P < 0.05$ compared to FD-Veh. **$P < 0.01$ compared to FD-Veh.

DISCUSSION

While activation of D1Rs and D2Rs had opposing effects on spontaneous myopia progression in albino guinea pigs,[17] it does not necessarily follow that this finding is generalizable to experimentally induced (FD) myopia in normal pigmented guinea pigs. Therefore, we performed a parallel study in pigmented animals to determine the corresponding mechanism(s). This undertaking was prompted by uncertainty as to whether albino eyes harbor structural and functional abnormalities that could interfere with the control of eye growth and myopia progression, abnormalities that presumably are absent in pigmented animals. Interestingly, we found here that the effects of DR-selective drugs in pigmented guinea pigs were very similar to those reported previously in albinos—namely, that the D1R agonist SKF38393 and the D2R antagonist sulpiride inhibited FDM, while the D2R agonist quinpirole promoted this response. In addition, the current study suggests that the DR drugs induced these effects through direct interactions with retinal D1Rs and D2Rs, rather than changes in DA release or turnover, since none of these drugs changed the DOPAC/DA ratios in the retina or vitreous after 2 weeks of treatment (these drugs are assumed to reach the retina during this period, given that APO injected into the subconjunctival space, comparable to the peribulbar space, reached a maximum concentration in the vitreous after 0.5 hour[5]). The equivalence of effects of these DA analogs on spontaneous myopia and FDM supports a balanced interaction model for eye growth regulation in both albino and pigmented animals, even though the latter do not spontaneously develop myopia.

It is noteworthy that in a normal visual environment (no FD), emmetropization was not altered, either by APO in mice,[11] or by L-DOPA in pigmented guinea pigs.[7] Similarly, in the present study, none of the selective DA analogs affected emmetropization of non-FD eyes in pigmented guinea pigs. However, these drugs altered spontaneous myopia in albino guinea pigs, as well as FDM in their pigmented counterparts. It

is possible that during exposure of pigmented guinea pigs to a normal visual environment, the balance between D1R and D2R activity maintains normal ocular growth. On the other hand, retinal DA levels declined, in both albino guinea pigs undergoing spontaneous myopia and pigmented guinea pigs exposed to FD. Under these circumstances, it is possible that D2R remains more strongly activated than D1R, as the former has a higher affinity for DA than the latter.[27]

Retinal and vitreous DOPAC/DA ratios vary from 0.1 to 0.3 in mice, monkeys, and chickens.[2] We reported in 2011 that this ratio was approximately 0.4 in pigmented guinea pigs,[5] while it was approximately 1 in the current study. It is possible that this marked difference in DOPAC/DA ratios, between pigmented guinea pigs and other animals studied to date, is due to a difference in sample collection methods. Previously, the retinal samples were collected under dim light, which may have led to inclusion of DA from the choroidal debris.[8] To minimize choroidal contamination in the current study, retinal samples were collected in a well-lighted room rather than under dim light. However, increasing the light intensity and spending additional time to remove choroidal debris may lower DA levels below those previously reported, because light and air are detrimental to the stability of DA.[28] In summary, potential factors contributing to the relatively high DOPAC/DA ratios observed in the current study are: (1) physiological increase in DA turnover, caused by brighter illumination immediately before sacrificing the animals; (2) lower DA levels resulting from less choroidal DA contamination, and (3) increased DA degradation induced by light and air. Despite the similarity between retinal DA and DOPAC levels, the significant declines in retinal DA and DOPAC levels induced by FD are consistent with those described in previous reports, suggesting that our sampling technique was valid. Meanwhile, although most studies have focused on retinal DA functions, it remains an open question as to whether or not also measuring choroidal DA release will lead to a different conclusion regarding retinal DA receptor involvement in controlling refractive development.

The opposing effects of D1R and D2R on spontaneous myopia development in albino guinea pigs and FDM in their pigmented counterpart support our "push-pull" balanced interaction working model describing DR involvement in controlling refractive error development (i.e., D1R activation inhibits myopic refractive error development, while D2R activation counters this effect). This push-pull paradigm is consistent with the opposing effects of D1R and D2R activation on adenylate cyclase activity. Specifically, D1R stimulates adenylate cyclase activity, whereas D2R inhibits it, suggesting that cAMP-sensitive responses could mediate either increases or decreases in myopia progression. Such opposing effects of D1R and D2R activation in other species are also reported to affect respiratory control,[29–31] licking behavior,[32] alcohol consumption,[33] etc.

There is an appreciable disparity in how DR subtypes mediate control of FDM in different animal models. McCarthy et al.[34] reported that intravitreal injection of the D1R agonist SKF38393 had no effect on FDM in chickens, whereas in the current study, peribulbar injection of SKF38393 suppressed FDM. The D1R antagonist SCH23390 has been used in numerous other studies, but its effects on FDM were also not consistent. D1R involvement in controlling myopia in chickens is questionable, because SCH23390 failed to reduce the protective effect of APO injections on FDM.[34–37] On the other hand, in chickens, intravitreal SCH23390 injection enhanced FDM in one study,[38] but had no effect in another study.[36] Also in chickens, unlike both albino and pigmented guinea pigs, D2R activation inhibited rather than promoted FDM: intravitreal injection of the D2R agonist quinpirole in chickens inhibited[34] FDM, whereas sulpiride, the D2R antagonist, enhanced it.[38]

Although reports of species-specific modes of D1R and D2R regulation are not novel as exemplified in guinea pig and chickens, it is also possible that drug-related differences in effects on FDM are attributable to differences in the route of drug delivery. Intravitreally injected DR drugs are expected to first reach and buildup in the retina; while if they are injected into the peribulbar space, it is likely that they reach the sclera, choroid, and RPE before the retina. Therefore, it is likely that there are different levels of drug plateaus in sclera, choroid, RPE, and retina following corresponding delivery route. Accordingly, intravitreal injections initially induce effects in the retina, whereas peribulbar injections cause drug effects preferentially by interactions with nonretinal DRs. Choroidal DR involvement is tenable, in chickens, because transient increases occurred in choroidal thickness 3 hours after intravitreal injections of APO and quinpirole,[39] although their inhibition of lens-induced myopia progression counters our observation of their stimulation of FDM in guinea pigs, which could be due to differences in myopia mechanism and species.

Taken together, data from the current study show that the effects of selective D1R and D2R agonists and antagonists on FDM in pigmented guinea pigs are consistent with those previously reported in albino guinea pigs. This agreement strengthens support for our balanced D1R/D2R push-pull interactive model as an explanation for how DA modulates eye growth and myopia through interactions with these two DR subtypes.

Acknowledgments

The authors thank Yongmei Zhong's team (Fudan University) for DOPAC quantification by HPLC, Guangyun Mao (Wenzhou Medical University) for his advice in statistics, Jiang-Fan Chen (Wenzhou Medical University) for his advice in interpreting pharmacological results, and William K. Stell (the Cumming School of Medicine, University of Calgary) who generously provided his recognized expertise in myopia research to edit and improve the manuscript content.

Supported by Grants 81371047 and 81422007 from the National Natural Science Foundation of China; Grants LZ14H120001 and LQ13H120005 from Natural Science Foundation of Zhejiang Province; Grant 2011CB504602 from the National Basic Research Program of China (973 Project); the Zhejiang Provincial Program for the Cultivation of High-Level Innovative Health Talents; and the National Young Excellent Talents Support Program.

Disclosure: **S. Zhang**, None; **J. Yang**, None; **P.S. Reinach**, None; **F. Wang**, None; **L. Zhang**, None; **M. Fan**, None; **H. Ying**, None; **M. Pan**, None; **J. Qu**, None; **X. Zhou**, None

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