Published in final edited form as:

Eur J Neurosci. 2015 January ; 41(1): 17–30. doi:10.1111/ejn.12783.

Dopamine D2 receptors preferentially regulate the development of light responses of the inner retina

Ning Tian¹, Hong-ping Xu², and Ping Wang¹

¹Department of Ophthalmology and Visual Science, University of Utah School of Medicine, Salt Lake City, UT 84132, USA

²Department of Neurobiology, Yale University School of Medicine, New Haven, CT 06520, USA

Abstract

Retinal light responsiveness measured via electroretinography undergoes developmental modulation and is thought to be critically regulated by both visual experience and dopamine. The primary goal of this study is to determine whether the dopamine D2 receptor regulates the visual experience-dependent functional development of the retina. Accordingly, we recorded electroretinograms from wild type mice and mice with a genetic deletion of the gene that encodes the dopamine D2 receptor raised under normal cyclic light conditions and constant darkness. Our results demonstrate that mutation of the dopamine D2 receptors preferentially increases the amplitude of the inner retinal light responses evoked by high intensity light measured as oscillatory potentials in adult mice. During postnatal development, all three major components of electroretinograms, the a-wave, b-wave and oscillatory potentials, increase with age. Comparatively, mutation of the dopamine D2 receptors preferentially reduces the age-dependent increase of b-waves evoked by low intensity light. Light deprivation from birth reduces the amplitude of b-waves and completely diminishes the increased amplitude of oscillatory potentials. Taken together, these results demonstrate that the dopamine D2 receptor plays an important role in the activity-dependent functional development of the mouse retina.

Keywords

Dopamine D2 receptor; electroretinogram; retinal development; activity-dependent plasticity; light deprivation

INTRODUCTION

The synaptic circuitry in the retina undergoes developmental modification in mammals, including humans. Visual experience affects many aspects of the functional and morphological development of the retina, including synaptic density, bipolar cell structure, expression of neurotransmitter receptors, retinal ganglion cell (RGC) synapses and dendrites (Xu and Tian, 2004; Tian, 2008; 2011). However, little is known about the mechanisms by which activity regulates the development of the retina. Dopamine receptors have been

thought to play important roles in the activity-dependent synaptic plasticity in the central nervous system (CNS) (Chergui, 2011; Edelman and Lessmann, 2013; Herwerth et al., 2012; Smith et al., 2005; Sun et al., 2005; Surmeier et al., 2007, 2010, 2011; Wolf, 2010; Xing et al., 2010; Xu and Yao, 2010; Zhu et al., 2012). In the retina, dopamine receptors are expressed by all types of neurons and are thought to regulate retinal development, synaptic formation, synaptic transmission, and light adaptation (Dowling, 2012; Nguyen-Legros et al., 1999). For instance, dopamine D1 receptors are expressed by horizontal cells and AII amacrine cells to regulate gap junction connections between these cells (Kothmann et al., 2009; Mills et al., 2007; Mills and Massey, 1995; Urschel et al., 2006; Zhang et al., 2011). Dopamine D1 receptors also regulate γ -aminobutyric acid (GABA) release from horizontal cells (Herrmann et al., 2011), acetylcholine release from amacrine cells (Hensler and Dubocovich, 1986; Hensler et al., 1987), light adaptation and sensitivity of RGCs (Van Hook et al., 2012; Vaquero et al., 2001), and are thought to regulate neurite outgrowth (Lankford et al., 1987) and activity-dependent ocular growth (Stone et al., 1990). Recently, we found that dopamine D1 receptors regulate the activity-dependent development of the mouse retina (He et al., 2013).

On the other hand, dopamine D2 receptors are expressed by photoreceptors (Ribelayga et al., 2008; Witkovsky et al., 1988), RGCs (Mills et al., 2007) and amacrine cells (Derouiche and Asan, 1999; Nguyen-Legros et al., 1999; Weber et al., 2001). Activation of dopamine D2 receptors regulates gap junction couplings between the rod-cone and gap junction couplings between RGCs (Mills et al., 2007; Ribelayga and Mangel, 2010), transmembrane currents in rods (Kawai et al., 2011), the amplitudes of electroretinogram (ERG) b-waves (Miranda-Anaya et al., 2002) and oscillatory potentials (OPs) (Perry and George, 2007), and the light responses of RGCs (Bodis-Wollner and Tzelepi, 1998). In addition, development and visual activity regulates the expression of dopamine receptors, the number of dopaminergic cells (Klitten et al., 2008; Melamed et al., 1986), and the storage and release of dopamine (Lorenc-Duda et al., 2009; Melamed et al., 1986; Shelke et al., 1997; Spira and Parkinson, 1991). Therefore, it is highly likely that the dopamine D2 receptor might also regulate the activity-dependent development of the retina.

To test this possibility, we examined the ERG of wild type (WT) mice and mice with mutation of gene encoding the dopamine D2 receptor (D2^{-/-} mice) raised under normal cyclic light/dark conditions and constant darkness. Our results demonstrate that dopamine D2 receptors play an important role in the activity-dependent functional development of the mouse retina.

EXPERIMENTAL PROCEDURES

Ethic Statement

All procedures for handling, maintenance and preparation of animals met the NIH guidelines for care and use of animals in research and were approved by the Animal Care and Use Committee of Yale University.

Animals and dark rearing

The procedures for animal handling and preparation in this study have been described previously (He et al., 2013; Vistamehr and Tian, 2004). ERGs were recorded from both the left and right eyes simultaneously of C57BL/6 (WT), D2^{-/-} and D3^{-/-} mice (The Jackson Laboratory, Bar Harbor, Maine). These mice were either raised under cyclic light/dark conditions as controls or under constant darkness. The control animals were fed and housed under 12:12 hour cyclic light/dark conditions in regular mouse rooms located in the Animal Care Facility. The average light intensity illuminating the cages during subjective day was 40 lux for control mice. Dark reared animals were housed in conventional mouse cages, which were placed in a continuously ventilated light-tight box. The temperature and humidity inside the box were continuously monitored and balanced by adjusting the speed of the ventilating fan. The box was placed in a light-tight room located in the same facility as control animals. All the procedures of daily monitoring and routine maintenance of dark reared mice were conducted under infrared illumination by trained personnel with the use of a pair of IR sensitive goggles (B.E. Meyers and Co. Inc., Redmond, WA).

ERG recordings and data analysis

The procedure of ERG recordings have also been described previously (He et al., 2013; Vistamehr and Tian, 2004) and fits the recommendations by the International Society for Clinical Electrophysiology of Vision (ISCEV) (Marmor et al., 2009). Briefly, animals reared under cyclic light/dark conditions were dark-adapted for at least 30 minutes before experiments. Dark reared mice were transferred from the dark room to the ERG recording room in a light-tight transfer box. Prior to the recordings, mice were anesthetized with Xylazine (13 mg/kg) and Ketamine (87 mg/kg) and the pupils were dilated with Atropine (1 %, Bausch & Lomb, Pharmaceuticals, Inc., Tampa, FL) and Phenylephrine HCl (Mydrin 2.5%, Alcon Inc., Humacao, Puerto Rico). A topical anesthetic agent, proparacaine (0.5 %, Alcon Inc., Humacao, Puerto Rico) was used before the contact electrodes were applied to the corneas. ERGs were evoked by 100 ms white flashes generated by LED arrays built in to a pair of miniature Ganzfeld stimulators for both the left and right eyes of each mouse (EPIC-3000, LKC Technologies Inc., Gaithersburg, MD) and recorded simultaneously for both eyes. Signals were band-pass filtered between 0.3 Hz to 500 Hz. For each of the intensities between 0.008 cd*s/m² and 0.8 cd*s/m², ERGs were averaged from 5 single flashes. Inter-stimulus interval was 30 seconds. ERGs were averaged from 3 single flashes for the intensities between 2.5 cd*s/m² and 25 cd*s/m². The inter-stimulus interval was 60 seconds. All recordings were made at approximately the same time of the day (from 11:00 AM to 3:00 PM).

Responses of ERG components were fitted to the following equation (a modified Naka-Rushton function) using software Igor (WaveMetrics, Lake Oswego, OR) with the Levenberg-Marquardt algorithm to determine the $R_{\max}$ and I_{50} :

$$R = R_{\min} + \frac{R_{\max} - R_{\min}}{1 + \left(\frac{I_{50}}{I}\right)^n}$$

Here R is the amplitude of ERG a-, b-wave or OPs, $R_{\max}$ is the amplitude of the saturated responses predicted from the recordings, $R_{\min}$ is the amplitude of the minimum responses predicted from the recordings, I is the light intensity for each recorded data point (R), I_{50} is the light intensity at which the half saturated response would be predicted from the recordings (semisaturation constant), and n is a variable that determines the steepness of the curves.

Analysis of variance (ANOVA), *post hoc* (Bonferroni/Dunn) test and analysis of covariance (ANCOVA) were used to determine the significance of the difference between more than two means and the interaction between two independent factors. Student t-tests were used to examine the difference between two means. All of the statistical tests were performed using software StatView (Abacus Concepts, Berkeley, CA).

RESULTS

The amplitude of inner retinal light responses is selectively enhanced in D2^{-/-} mice

The dopamine D2 receptors are found to be expressed in both the inner and outer retina (Derouiche and Asan, 1999; Kothmann et al., 2009; Mills et al., 2007; Nguyen-Legros et al., 1999; Ribelayga et al., 2008; Van Hook et al., 2012; Vaquero et al., 2001; Weber et al., 2001; Witkovsky et al., 1988; Zhang et al., 2011) but the effects of dopamine D2 receptors on ERG are contradictory. Pharmacological blockade or genetic mutation of dopamine D2 receptors have been shown to increase the amplitudes of b-waves in goldfish and cats and the OPs of mice ERG (Kim and Jung, 2012; Lavoie et al., 2013; Schneider and Zrenner 1991) but decrease the amplitude of ERG b-wave in rabbits (Huppé-Gourgues et al., 2005). On the other hand, activation of dopamine D2 receptor enhances the amplitudes of b-wave of scotopic ERG in green iguana retina (Miranda-Anaya et al., 2002) but had no effect on rabbit ERG (Huppé-Gourgues et al., 2005). We first determined the effects of genetic mutation of the dopamine D2 receptor on the ERG responses in young adult mice. ERGs responding to 8 different light intensities were recorded from WT, D2^{-/-} and D3^{-/-} mice at the age of postnatal day 30 (P30). WT mice were used as negative controls, as were D3^{-/-} mice. Because the retina does not express dopamine D3 receptors (Jackson et al., 2009) and mutation of dopamine D3 receptor has no detectable effect on ERG b-wave (Hermann et al., 2011), it allows us to use the D3^{-/-} mice as controls for the effects of non-retinal dopamine signaling defects. The amplitudes of the three major components of ERG, a-wave, b-wave and OPs, were plotted as a function of stimulating light intensity. Fig 1A shows representative waveforms of ERGs (left) and OPs (right) recorded from a WT and a D2^{-/-} mouse. The initial portion of the a-wave is a measurement of photoreceptor function. The b-wave is mainly a measurement of ON bipolar cell function (Stockton & Slaughter, 1989; Tian & Slaughter, 1995). The OPs, which reflects interactions among bipolar, amacrine and ganglion cells, is a measurement of inner retinal function (Wachtmeister, 1998). The OPs shown in Fig 1A were isolated by band-pass filtering (73 Hz to 500 Hz) the waves of ERGs and the amplitudes of OPs were calculated by the sum of all peaks (Severns *et al.*, 1994). The light intensities used to evoke ERG responses cover a wide range of luminosity from 0.008 to 25 cd*s/m². This will only stimulate rods at the lower intensities and stimulate both rods and cones at the high intensities. Because no background light was used to isolate cone-

mediated light responses, the ERGs recorded in this study are either scotopic or mesopic responses.

Figs 1B, 1D and 1F show the average intensity-response curves of ERG a-wave, b-wave and OPs recorded from WT, D2^{-/-} and D3^{-/-} mice. For a-wave and OPs, the average intensity-response curves of WT, D2^{-/-} and D3^{-/-} mice all have sigmoid distribution, indicating that the light stimuli cover most of the dynamic range of photoreceptors and inner retinal light responses. On the other hand, the average intensity-response curves of b-waves of all three strains of mice have a somewhat linear distribution, indicating that the range of light intensity was probably only wide enough to cover the middle portion of the whole dynamic range of bipolar cell light responses. By comparing the intensity-response curves of a- and b-wave in D2^{-/-} mice with WT controls, it is evident that the amplitudes of ERG a- and b-wave are not affected by the mutation of dopamine D2 receptors at most of the light intensities except the lowest light intensity (Figs 1B and 1D). However, the amplitudes of OPs evoked by high intensity light in D2^{-/-} mice are significantly higher than that of WT controls (Fig 1F). This is consistent with a recent report (Lavoie et al., 2013). As expected, mutation of the dopamine D3 receptors has no effect on any of the ERG components.

To further reveal the relative extent of the alternations of ERG responses due to D2 receptor mutation, we quantified the differences of the intensity-response curves of ERG a-, b-wave and OPs between D2^{-/-} and WT mice by normalized the responses of D2^{-/-} mice to the age-matched WT controls using the following equation:

$$R_{\text{nor}}(i) = R(i) / R_{\text{ave}}(i) \times 100$$

Here $R_{\text{nor}}(i)$ represents the normalized a-, b-wave or OPs amplitudes of D2^{-/-} mice evoked by light intensity (i). $R(i)$ represents the actual a-, b-wave or OPs amplitudes of D2^{-/-} mice evoked by light stimulus (i). $R_{\text{ave}}(i)$ is the average amplitudes of a-, b-wave or OPs evoked by light stimulus (i) of WT controls. Therefore, the results of D2^{-/-} mice are expressed as percentiles of the responses of WT mice. For the ERG a-waves of D2^{-/-} mice, only the normalized amplitude for the lowest light intensity (0.008 cd*s/m²) was increased to 226% ± 60.3% of WT controls (Fig 1C). For b-waves, the normalized amplitude of the response to the lowest light intensity was reduced to 71% ± 4.7% of WT controls (Fig 1E). The amplitudes of a- and b-waves of D2^{-/-} mice evoked by other light intensities are not different from that of WT controls. In contrast from the ERG a- and b-waves, the normalized OPs amplitudes of D2^{-/-} mice have biphasic changes in comparison with the WT controls. At the light intensities of 0.008 cd*s/m² and 0.025 cd*s/m², the normalized OPs amplitudes were reduced to 80% ± 15.2% and 69% ± 10.7% of WT controls while the normalized OPs amplitudes evoked by the lights of 2.5 cd*s/m², 8 cd*s/m² and 25 cd*s/m² were increased to 138% ± 14.6%, 129% ± 8.9% and 125% ± 8% of WT controls (Fig 1G).

We further examined this data by fitting it to a modified Naka-Rushton function (see detailed description in Methods) to predict the maximal a-, b-wave and OPs responses and the hemisaturation constant (I_{50}) of D2^{-/-} and WT mice. Consistent with the intensity-response curves, the predicted maximal a- and b-wave amplitudes of D2^{-/-} mice are not significantly different from that of WT controls (Table 1). The average maximal a-wave

amplitudes are $-446.9 \pm 37.4 \mu\text{V}$ versus $-445.2 \pm 18 \mu\text{V}$ (mean $\pm$ SE for these and all following expressions, $p = 0.8955$) and the average maximal b-wave amplitudes are $1046.3 \pm 75.9 \mu\text{V}$ versus $1108.1 \pm 44 \mu\text{V}$ for D2 $^{-/-}$ and WT mice, respectively ($p = 0.7029$). Although the average maximal OPs amplitude of D2 $^{-/-}$ mice is 17.7% higher than that of WT controls ($1076.4 \pm 73.9 \mu\text{V}$ versus $914.3 \pm 44.4 \mu\text{V}$), the difference is not statistically significant ($p = 0.0664$, Table 1). The I_{50} for a-, b-wave and OPs of D2 $^{-/-}$ mice are not statistically different from that of age matched WT controls (data not shown).

The differences in the changes of the amplitudes of a-, b-wave and OPs of D2 $^{-/-}$ mice imply that dopamine D2 receptors might regulate the light response gain differently in the inner and outer retina. To further examine this possibility, we assessed the changes of response gains of outer and inner retina due to D2 receptor mutation by comparing the ratio of b/a-wave and the ratio of OPs/b-wave of D2 $^{-/-}$ and WT mice. When the average b/a-wave ratios of WT mice were plotted as a function of light intensity (Fig 2A), the curve is relatively flat with the highest b/a-wave ratio (-24.4 ± 5.45) at the light intensity of $0.025 \text{ cd}^*\text{s}/\text{m}^2$ and the lowest b/a-wave ratio (-2.14 ± 0.13) at the light intensity of $25 \text{ cd}^*\text{s}/\text{m}^2$. These results demonstrate that the strength of light response gain at the outer retina depends upon the intensity of light stimulation and the light responses evoked by weaker light stimuli have stronger synaptic gain. However, the D2 $^{-/-}$ mice seem to have a highly elevated b/a-wave ratio at light intensity of $0.025 \text{ cd}^*\text{s}/\text{m}^2$. The average b/a-wave ratios of WT and D2 $^{-/-}$ mice at the light intensity of $0.025 \text{ cd}^*\text{s}/\text{m}^2$ are -24.41 ± 5.45 and -67.45 ± 23.2 respectively but the difference is not statistically significant ($p = 0.1692$).

To further reveal the relative extent of the alternations of ERG responses gain due to D2 receptor mutation, we normalized the response gain of D2 $^{-/-}$ mice to the age-matched WT controls using the following equation:

$$G_{\text{nor}}(i) = G(i) / G_{\text{ave}}(i) \times 100$$

Here $G_{\text{nor}}(i)$ represents the normalized response gain of D2 $^{-/-}$ mice evoked by light intensity (i). $G(i)$ represents the actual response gain of D2 $^{-/-}$ mice evoked by light stimulus (i). $G_{\text{ave}}(i)$ is the average response gain evoked by light stimulus (i) of WT control mice. Therefore, the results of D2 $^{-/-}$ mice are expressed as percentile of the response gain of WT mice. Fig 2B shows that the b/a-wave ratio of D2 $^{-/-}$ mice at the light intensity of $0.025 \text{ cd}^*\text{s}/\text{m}^2$ is $276.3\% \pm 95\%$ of that of WT controls. In contrast, the ratios of OPs/b-wave of D2 $^{-/-}$ mice also have biphasic changes with the OPs/b-wave ratio at light intensity of $0.025 \text{ cd}^*\text{s}/\text{m}^2$ decreased to $75.9\% \pm 12.2\%$ of WT controls ($p = 0.0368$), while the OPs/b-wave ratios at light intensities of $0.8 \text{ cd}^*\text{s}/\text{m}^2$, $2.5 \text{ cd}^*\text{s}/\text{m}^2$, $8 \text{ cd}^*\text{s}/\text{m}^2$ and $25 \text{ cd}^*\text{s}/\text{m}^2$ increased to $126.6\% \pm 16\%$, $131\% \pm 14.6\%$, $128.4\% \pm 11.3\%$ and $124.8\% \pm 8.8\%$ of WT controls ($p = 0.0381$, 0.0229 , 0.0009 and 0.0022 , Fig 2D). These results support the notion that activation of the dopamine D2 receptor preferentially decreases the transmission of visual signaling evoked by low intensity light in the outer retina and inversely increases the transmission of visual signaling evoked by low intensity light in the inner retina. In addition, activation of D2 receptors selectively decreases the transmission of visual signaling evoked by high intensity light in the inner retina without affecting the outer retinal responses to high

intensity light. Furthermore, we examined the light response kinetics of ERG by measuring the peak times of ERG a-waves (Fig 2E) and b-waves (Fig 2F) of both D2^{-/-} and WT mice. We found minimal differences between WT and D2^{-/-} mice in the peak time for both a- and b-waves.

Overall, the most significant effect of dopamine D2 receptor mutation on the retinal light responses is the increase in the amplitude of OPs to high intensity light, which is the opposite effect observed when the dopamine D1 receptor is mutated (He et al., 2013; Lavoie et al., 2013). On the other hand, mutation of the dopamine D2 receptor increases the light response gain of the outer retina and decreases the light response gain of the inner retina to low intensity light, which is similar to the effect induced by mutation of the dopamine D1 receptor (He et al., 2013). Although the changes in the response gain of the inner and outer retina to low intensity light occur in opposite directions, the magnitude of the amplitude changes in a-wave and OPs to low intensity light is not as significant as the changes of OPs to high intensity light. Despite this, the relative impact is still strong and significant.

Mutation of dopamine D2 receptor reduces the developmental increase of ERG b-wave amplitudes

It has been shown that the amplitudes of ERG undergo postnatal developmental enhancement in humans and other mammals (Ben-Shlomo and Ofri, 2006; Breton et al., 1995; el Azazi and Wachtmeister, 1991a; 1991b; Flores-Guevara et al., 1996; Gorfinkel et al., 1988; Reuter, 1976; Rodriguez-Saez et al., 1993; Sandalon and Ofri, 2012; Westall et al., 1999), and mutation of the dopamine D1 receptor reduces the developmental increase of the ERG b-waves (He et al., 2013). To determine whether mutation of the dopamine D2 receptor impacts the development of light responsiveness of the retina, we recorded the ERG from WT and D2^{-/-} mice at the time of eye opening (P13) and compared with the ERGs from young adults (P30).

Figs 3A, 3C and 3E show the average intensity-response curves of ERG a-, b-wave and OPs recorded from D2^{-/-} and WT mice at the age of P13, respectively. Similar to the P30 mice, the average intensity-response curves of a-wave and OPs of both WT and D2^{-/-} mice have a sigmoid distribution, while the average intensity-response curves of b-waves of both WT and D2^{-/-} mice have a linear distribution. The average amplitudes of ERG a-wave of P13 D2^{-/-} mice are not statistically different from that of age-matched WT controls in most light intensities (Fig 3A) except during one light intensity. Although the average amplitudes of ERG b-wave and OPs of P13 D2^{-/-} mice seem to be systematically higher than that of age-matched WT controls but the differences at most of the light intensities are not statistically significant (Figs 3C and 3E). The predicted maximal amplitude of a-waves is reduced in D2^{-/-} mice but the predicted maximal amplitudes of b-waves and OPs of D2^{-/-} mice are increased in comparison with the age-matched WT controls. The differences between the predicted maximal values of ERG a-wave and OPs of WT and D2^{-/-} mice are statistically significant (Table 1). These results support the idea that mutation of D2 receptor has detectable effects on the light responsiveness of both the inner and outer retina at the early stage of postnatal development.

We then determined whether mutation of the dopamine D2 receptor affects the developmental changes of ERG responses after eye-opening. We first calculated the ratios of ERGs recorded at P13 and P30 to determine the magnitude of developmental changes of the major components of ERGs in WT and D2^{-/-} mice, and then compared the developmental changes of D2^{-/-} mice with WT controls. Fig 3B shows the P30/P13 ratios of ERG a-wave amplitudes in WT and D2^{-/-} mice as a function of light intensity. Although with significant variation, it is evident that the P30/P13 ratios of a-wave amplitudes are greater than 100% at most light intensities for both WT and D2^{-/-} mice except a few intensities (from $38.9\% \pm 10\%$ and $181.8\% \pm 41.3\%$ at $0.008 \text{ cd}^*\text{s/m}^2$ to $718.4\% \pm 18.7\%$ and $544.7\% \pm 34.3\%$ at $25 \text{ cd}^*\text{s/m}^2$ for WT and D2^{-/-} mice, respectively), indicating a developmental increase of the ERG a-wave amplitude for most of the tested light intensities. In addition, the P30/P13 ratio increases with the light intensity for both WT and D2^{-/-} mice, indicating a stronger developmental enhancement of light responsiveness mediated by cones. Furthermore, the P30/P13 ratios of ERG a-wave amplitudes of WT and D2^{-/-} mice have a similar distribution pattern at most light intensities and the distribution curves are not systematically different except a few sporadic points of light intensities where either WT or D2^{-/-} mice have a higher ratio. These results demonstrate that the a-wave amplitudes increase from P13 to P30 for both WT and D2^{-/-} mice and mutation of dopamine D2 receptor has limited effect on the maturation of photoreceptor light response.

Similar to the ERG a-wave, the P30/P13 ratios of ERG b-wave are also greater than 100% at all light intensities for both WT and D2^{-/-} mice (from $676.1\% \pm 21.2\%$ and $396.3\% \pm 26.5\%$ at $0.008 \text{ cd}^*\text{s/m}^2$ to $423.1\% \pm 10.6\%$ and $376.4\% \pm 19.2\%$ at $25 \text{ cd}^*\text{s/m}^2$ for WT and D2^{-/-} mice, respectively) (Fig 3D), demonstrating that the amplitudes of b-wave increased by approximately 4–7 fold from P13 to P30 for both WT and D2^{-/-} mice. As shown previously, the P30/P13 ratio of ERG b-wave of WT mice decreases with the increase of light intensity (He et al., 2013), indicating a stronger developmental enhancement of the response gain between rods to rod-bipolar cells in comparison with the response gain between cones to cone-bipolar cells. Interestingly, not only are the P30/P13 ratios of b-wave amplitudes of D2^{-/-} mice significantly lower than that of WT controls at all light intensities, but the light intensity-dependent decrease of the P30/P13 ratios of b-wave is also diminished in the D2^{-/-} mice. This is similar to the effects observed from D1^{-/-} mice (He et al., 2013) and these results strongly suggest that both D1 and D2 receptors preferentially regulate the developmental increase of response gain between rods and rod-bipolar cells.

Fig 3F shows the P30/P13 ratios of OPs amplitudes of WT and D2^{-/-} mice as a function of light intensity. Again, the P30/P13 ratios of OPs amplitudes of WT and D2^{-/-} mice are greater than 100% at all light intensities (from $136.9\% \pm 7.9\%$ and $125.6\% \pm 23.8\%$ at $0.008 \text{ cd}^*\text{s/m}^2$ to $804\% \pm 32.9\%$ and $661\% \pm 42.5\%$ at $25 \text{ cd}^*\text{s/m}^2$ for WT and D2^{-/-} mice, respectively). The distributions of the P30/P13 ratios of OPs amplitudes of both WT and D2^{-/-} mice show a biphasic pattern, in which the P30/P13 ratio of WT mice sharply increases with light intensities from $0.008 \text{ cd}^*\text{s/m}^2$ to $0.08 \text{ cd}^*\text{s/m}^2$ and reach a relatively flat phase at light intensities between $0.08 \text{ cd}^*\text{s/m}^2$ and $25 \text{ cd}^*\text{s/m}^2$. However, the D2^{-/-} mice have a clearly reduced sharpness of the light intensity-dependent increase of the P30/P13 ratios of OPs amplitudes between $0.008 \text{ cd}^*\text{s/m}^2$ to $0.25 \text{ cd}^*\text{s/m}^2$. By comparing the OPs amplitudes of mice at P13 and P30 (Figs 1F and 3F), this seems to be the result of a

slight enhancement of OPs amplitude to the low-intermediate light intensities of D2^{-/-} mice. These results suggest that activation of dopamine D2 receptors suppresses the inner retinal light responses in both young and adult mice but has more prominent effects in adult mice.

Dopamine D2 receptors interact with visual activity to regulate ERG

The development of the mouse retinal light responses is sensitive to light deprivation (Tian and Copenhagen, 2003; Vistamehr and Tian, 2004; He et al., 2013) and the dopamine D1 receptor has been shown to regulate the activity-dependent development of retinal light responses measured as ERG (He et al., 2013). Therefore, we further investigated whether the dopamine D2 receptor also participates in the activity-dependent development of retinal light responses using ERG recordings. Accordingly, we dark reared both D2^{-/-} and WT mice from birth to P30 and compared the ERG responses of these dark reared mice with that of age-matched controls raised under cyclic light conditions. As reported previously (Vistamehr and Tian, 2004; He et al., 2013), WT mice raised in constant darkness from birth to P30 have reduced amplitudes of all three major components of ERG, especially the amplitudes of b-waves and OPs in response to high intensity light (Figs 4B and 4C). Similar to the WT mice, the amplitudes of a-, b-waves and OPs of dark reared D2^{-/-} mice are reduced (Figs 4E–4G). Interestingly, although mutation of the dopamine D2 receptor significantly increased the amplitudes of OPs (Fig 4D) to high intensity light, the amplitudes of OPs of dark reared D2^{-/-} mice are not different from that of age-matched WT mice raised in constant darkness at all light intensities (Fig 4H). These results suggest that the D2 receptor-dependent regulation of the amplitude of OPs is sensitive to light deprivation.

Because mutation of the dopamine D1 receptor differentially affects the response gain and kinetics of the inner and outer retina of dark reared mice (He et al., 2013), we further analyzed the b/a-wave ratio and the OPs/b-wave ratio of dark reared WT and D2^{-/-} mice and compared the results with that of D2^{-/-} and WT mice raised under cyclic light conditions. Fig 5A shows that light deprivation of WT mice significantly increased the b/a-wave ratios to low intensity light, while light deprivation of D2^{-/-} mice caused this ratio to slightly decrease, but not in a statistically significant manner (Fig 5C). This is similar to what occurs when the dopamine D1 receptor is mutated, but displays a much weaker magnitude (He et al., 2013). Fig 5E compares the effects of dark rearing on the b/a-wave ratios of WT and D2^{-/-} mice by normalizing the b/a-wave ratios of dark reared WT mice to the age-matched WT controls and the b/a-wave ratios of dark reared D2^{-/-} mice to the age-matched D2^{-/-} control mice. It is evident that dark rearing preferentially affects the b/a-wave ratio to low intensity light in WT mice, but with very little effect on D2^{-/-} mice, suggesting that the light-dependent change of response gain of outer retina is regulated by both dopamine D1 and D2 receptors. In the inner retina, dark rearing has very little effect on the OPs/b-wave ratio of WT mice (Fig 5B) but significantly reduces the OPs/b-wave ratio to high intensity light in D2^{-/-} mice (Fig 5D). The data for normalized OPs/b-wave ratios of dark reared WT and D2^{-/-} mice (Fig 5F) confirms the notion that light deprivation preferentially decreases the OPs/b-wave ratio of D2^{-/-} mice to high intensity light (Fig 5F). This is the opposite of the effect of mutating the dopamine D1 receptor, in which light deprivation increases the OPs/b-wave ratio for all light intensities (He et al., 2013).

Finally, we examined the effects of dark rearing on the light response kinetic of ERG by measuring the peak times of ERG a-wave and b-wave of both WT and D2^{-/-} mice raised in constant darkness and cyclic light conditions. In WT mice, dark rearing reduced the peak time of both a- and b-waves at most light intensities (Figs 6A and 6B). In D2^{-/-} mice, dark rearing had little effect on the peak times of both a- and b-wave (Figs 6C and 6D). When we compare the effects of dark rearing on the a- and b-wave peak times of WT and D2^{-/-} mice by normalizing the a- and b-wave peak times of dark reared mice to the genetic- and age-matched controls, it is evident that dark rearing induced effects on a-wave peak times of WT mice are light intensity dependent, in which the changes of the a-wave peak times in dark reared mice have a higher magnitude for responses evoked by low-intermediate light intensities except for the lowest intensity. However, this light intensity-dependent change of a-wave peak time is largely diminished in dark reared D2^{-/-} mice (Fig 6E). For the b-wave peak time, dark rearing had much weaker effect on both WT and D2^{-/-} mice and the changes across all the light intensities have a similar magnitude (Fig 6F).

Overall, these results demonstrate that light deprivation completely diminishes the enhancement of OPs amplitude induced by mutation of dopamine D2 receptor through suppression of the response gain of the inner retina. In addition, mutation of the dopamine D2 receptor diminishes the effects of dark rearing on the outer retinal response gain measured as the b/a-wave ratio. Furthermore, mutation of the dopamine D2 receptor reduces the effect of dark rearing on the response kinetics of both photoreceptors and bipolar cells.

DISCUSSION

The goal of this study is to determine whether the dopamine D2 receptor regulates the activity-dependent development of retinal light responsiveness different from that of the dopamine D1 receptor. Our results showed several findings which have not been previously reported. First, mutation of the dopamine D2 receptor preferentially increases the amplitude of OPs presumably through a cone-mediated pathway by enhancing the response gain of the inner retina. This increase of the amplitude of OPs is opposite to the effect induced by mutation of the dopamine D1 receptor. Second, mutation of the dopamine D2 receptor reduces the developmental increase of the amplitude of ERG b-wave after eye opening. This result is in the same direction as that of mutation of the dopamine D1 receptor. Third, light deprivation of D2^{-/-} mice reduces the amplitudes of ERG b-waves, which is similar to that of D1^{-/-} mice, but completely diminishes the increased amplitude of OPs due to the mutation of dopamine D2 receptors, which is somewhat opposite to that of D1^{-/-} mice. Taken together, these results demonstrate that the dopamine D2 receptors play important roles in the activity-dependent functional development of the mouse retina, and mutation of the dopamine D1 and D2 receptors results in similar functional defects in the outer retina light responses but opposite functional defects in the inner retina light responses.

The effects of D2 receptor on ERG of adult mice

In vertebrate retina, dopamine is released by dopaminergic amacrine cells and is known to alter the physiology of retinal cells. However, specific dopamine receptor subtypes regulate retina physiology differently. Several studies have investigated the effects of dopamine D2

receptors on the ERG with contradictory results. In most vertebrates, the amplitude of ERG b-wave varies with a circadian rhythm. The amplitude of ERG b-wave is high during the day and low during the night while dopamine levels are high during the day and low during the night (Miranda-Anaya et al., 2002). In the Japanese quail, blocking dopamine D2 receptors during the day increases the amplitude of ERG b-wave while activating D2 receptors at night decreases the amplitude of the b-wave (Manglapus et al., 1999). In contrast, activating dopamine D2 receptors during the night increases the amplitude of b-waves in the green iguana (Miranda-Anaya et al., 2002) and blocking dopamine D2 receptors in rabbits decreases the amplitude of ERG b-wave (Huppé-Gourgues et al., 2005). Further complicating matters the D2 antagonist, sulpiride, enhances the amplitude of the b-wave mediated by rods but diminishes the b-wave mediated by cones in dark adapted frogs (Popova and Kупenova, 2013). In the inner retina, activating dopamine D2 receptors increases the amplitude of OPs mediated by the rod pathway in the tiger salamander (Perry and George, 2007) and blocking of the dopamine D2 receptors decreases the amplitude of OPs in the mudpuppy (Wachtmeister and Dowling, 1978; Wachtmeister, 1981; Wachtmeister, 1998).

Our results seem to increase the complexity of the effects mediated by the dopamine D2 receptor in the retinal light responses. D2^{-/-} mice at P30 shows minimal change in the amplitudes of ERG a- and b-waves to most of the light intensities except for the lowest light intensity. At this lowest light intensity, D2^{-/-} mice have slightly increased amplitude of ERG a-wave and slightly reduced amplitude of ERG b-wave, which is similar to the effects mediated by D2 receptor antagonists on rabbit ERG (Huppé-Gourgues et al., 2005) but opposite of the observation from frogs (Popova and Kупenova, 2013) and Japanese quail (Manglapus et al., 1999). In the inner retina, D2^{-/-} mice have slightly reduced amplitude of OPs to low intensity light, which is similar to the reports in the mudpuppy (Wachtmeister and Dowling, 1978; Wachtmeister, 1981; 1998), but significantly increased amplitude of OPs to high intensity light, which is opposite of the results of low vertebrates (Perry and George, 2007; Wachtmeister and Dowling, 1978; Wachtmeister, 1981; 1998).

Although both D1 and D2 dopamine receptors are expressed by retinal neurons (Dowling, 2012; Nguyen-Legros et al., 1999), these two subtypes dopamine receptors are considered to have distinct features in many aspects (Bloomfield and Völgyi, 2009; Derouiche and Asan, 1999; DeVries and Schwartz 1989; Kothmann et al., 2008; 2009; Lasater et al., 1987; Mills and Massey, 1995; Mills et al., 2007; Nguyen-Legros et al., 1999; Ribelayga et al., 2002; 2008; Ribelayga and Mangel, 2010; Urschel et al., 2006; Weber et al., 2001; Witkovsky et al., 1988; Zhang et al., 2011). In the ERG, adult D1^{-/-} mice have reduced amplitudes of a-, b-wave and OPs (He et al., 2013; Herrmann et al., 2011; Jackson et al., 2012; Lavoie et al., 2013), while adult D2^{-/-} mice have minimal change in the amplitudes of ERG a- and b-waves but significant changes in the amplitude of OPs. These results support the idea that the dopamine D1 receptor regulates the light response of both the inner and outer retina while the dopamine D2 receptor preferentially regulates the light response of the inner retina. In addition, genetic mutation of the dopamine D2 receptor, the potential autoreceptor to control dopamine release from dopaminergic amacrine cells (Derouiche and Asan, 1999; Nguyen-Legros et al., 1999; Weber et al., 2001), does not seem to cause detectable over-excitation of the dopamine D1 receptors at the outer retina, while pharmacological over-

excitation of the dopamine D1 receptors profoundly increases the amplitudes of ERG a-, b-wave and OPs (Kim and Jung, 2012; Oliver et al., 1987; Wachtmeister and Dowling, 1978).

In the inner retina, D2^{-/-} mice have a biphasic change of the amplitude of OPs, a slightly reduced amplitude of OPs to low intensity light and a significantly increased amplitude of OPs to high intensity light. The increased OPs amplitude is consistent with a recent report of ERG of D2^{-/-} mice (Lavoie et al., 2013). Given the considerations that the OPs are likely to be generated by the synaptic activity between the bipolar cells, the amacrine cells, and the RGCs (Wachtmeister, 1998), and that both the dopamine D1 and D2 receptors are expressed by amacrine cells and RGCs (Derouiche and Asan, 1999; Kothmann et al., 2009; Mills et al., 2007; Mills and Massey, 1995; Nguyen-Legros et al., 1999; Urschel et al., 2006; Weber et al., 2001; Zhang et al., 2011), it is possible that both inactivation of dopamine D2 receptors and over-excitation of dopamine D1 receptors could attribute to the changes in the amplitude of OPs of D2^{-/-} mice. Consistently, activating dopamine D2 receptors increases the amplitude of OPs mediated by the rod pathway (Perry and George, 2007) and blocking of dopamine D1 receptors or mutation of dopamine D1 receptor reduces the amplitude of OPs (He et al., 2013; Holopigian et al., 1994; Lavoie et al., 2013). This possibility is further supported by the results that dark rearing D2^{-/-} mice, which could potentially block dopamine release from dopaminergic amacrine cells and dopamine D1 receptor activation, completely diminishes the effect of mutant the dopamine D2 receptors on the amplitude of OPs.

The effects of D2 receptor on ERG of developing mice

The changes of ERG during postnatal development have been widely reported and the ages at which ERG parameters reach adult values vary considerably across species. In human, the a- and b-waves appear at birth and the amplitudes of these waves increase considerably during postnatal development and reach the adult level by the ages of 3–15 years (Breton et al., 1995; Flores-Guevara et al., 1996; Rodriguez-Saez et al., 1993; Westall et al., 1999), while the a/b-wave ratio remains constant during postnatal development (Flores-Guevara et al., 1996), suggesting that the response gain at the outer retina is balanced and stabilized during the whole course of the postnatal development. On the other hand, the OPs are the most immature component of ERG in early infancy, but develop quickly and reach the adult level by two years of age (Westall et al., 1999). In guinea pig, the amplitudes of ERG detected at birth was 50% of the adult level and reached maximal values 12 days after birth (Bui and Vingrys, 1999). In rabbit and rat, the a-wave appears during the second postnatal week and the amplitude of a-wave reaches the adult value by the age of P30–40. After the a-wave, the b-wave and OPs appear and rapidly grow between the second and third weeks but continue to increase slowly after P40 (Braekevelt and Hollenberg, 1970; el Azazi and Wachtmeister, 1990; 1991a; 1991b; Gorfinkel and Lachapelle, 1990; Gorfinkel *et al.*, 1988; Masland, 1977; Reuter 1976; Weidman and Kuwabara, 1968, 1969).

We recent reported that all three major components of mouse ERG are detectable before the eye-opening (P10) and the amplitudes of these three components increase by up to 4–6 fold from P13 to P30. Interestingly, the age-dependent increase of ERG amplitudes is light intensity-dependent and the light intensity-dependency of a-, b-waves and OPs have

different patterns. The amplitudes of both ERG a-wave and OPs have much weaker age-dependent enhancement with low intensity light but much stronger age-dependent enhancement with high intensity light stimulation. On the other hand, the amplitude of the ERG b-waves has much stronger age-dependent enhancement with low intensity light but much weaker age-dependent enhancement with high intensity light. These results suggest that the maturation of the retina has a strong effect on cone-mediated responses at the photoreceptors and the inner retina but bipolar cells are influenced more through the rod-mediated responses (He et al., 2013). Therefore, the developmental profiles of retina vary significantly among different species of rodents and the increase of ERG amplitudes in postnatal development seems related to the maturation of retinal neurons (Bui and Vingrys, 1999; Hamasaki and Maguire, 1985; Tucker *et al.*, 1982).

We also found that dopamine D1 receptor selectively regulates the postnatal development of bipolar cell light responses by increasing the amplitude of ERG b-wave at P13 and decreasing the amplitude of ERG b-wave, especially the response evoked by high intensity light, at P30 and, therefore, diminishes the light intensity-dependent developmental increase in ERG b-waves. Surprisingly, the light intensity-dependent developmental increase of ERG b-wave is also diminished in the D2^{-/-} mice. Therefore, both dopamine D1 and D2 receptors seem to regulate the ERG b-waves in a similar manner, which appears to inhibit ERG b-waves during early postnatal development but enhances ERG b-waves in adulthood (He et al., 2013).

The role of dopamine D2 receptor on the activity-dependent developmental changes of ERG

The effects of visual experience on the developmental changes in ERG responses have been reported previously. Dark rearing or monocular light deprivation of cats for 2 to 4 weeks suppresses b-wave amplitudes (Baxter and Riesen, 1961; Babkoff, 1975). Dark rearing mice from birth to P30-90 decreases the amplitudes of ERG a-, b-waves and OPs (Vistamehr and Tian, 2004). In the inner retina, light deprivation induced suppression of the amplitudes of OPs can be completely reversed by returning the mice back to cyclic light/dark conditions for 1 to 2 weeks (Vistamehr and Tian, 2004). Although dopamine has been thought to regulate the activity-dependent synaptic plasticity in the CNS (Chergui, 2011; Edelman and Lessmann, 2013; Herwerth et al., 2012; Smith et al., 2005; Sun et al., 2005; Surmeier et al., 2007, 2010, 2011; Wolf, 2010; Xing et al., 2010; Xu and Yao, 2010; Zhu et al., 2012) and the synaptic formation, synaptic transmission, and light adaptations in the retina (Dowling, 2012; Lankford et al., 1987; Nguyen-Legros et al., 1999; Stone et al., 1990; Van Hook et al., 2012; Vaquero et al., 2001), little is known whether and how different subtypes of dopamine receptors regulate the activity-dependent development of retinal light responsiveness. Our recent studies showed that dark rearing of WT mice reduces the amplitudes of b-wave and OPs, increases the outer retinal light response gain to low intensity light, and alters the light response kinetics of both a- and b-waves (Tian and Copenhagen, 2003; Vistamehr and Tian, 2004; He et al., 2013). Mutation of the dopamine D1 receptor diminishes the effects of dark rearing on the amplitudes of OPs, reverses the dark rearing induced effects on the response gain of the outer retina, and the changes of the kinetics of ERG a-waves (He et al., 2013).

This demonstrates the multiple roles of dopamine D1 receptors in the activity-dependent functional development of the mouse retina.

In this study, our results show that dark rearing of D2^{-/-} mice has similar effects as that of dark rearing of D1^{-/-} mice on the light responses in the outer retina. Because the amplitudes of ERG b-wave are reduced by dark rearing in WT, D1^{-/-} and D2^{-/-} mice, it rules out the possibility that light deprivation suppresses the amplitudes of ERG b-wave through dopamine D1 or D2 receptors. In addition, genetic disruption of the gene for dopamine D4 receptors has no effect on the ERG b-wave of a dark adapted retina, although it reduced the amplitude of ERG b-wave during dark and light adaptation (Nir et al., 2002; Hermann et al., 2011). Therefore, it is unlikely that light deprivation suppresses the amplitude of the ERG b-waves through dopamine D4 receptors.

In the inner retina, dark rearing of D2^{-/-} mice completely diminishes the increase of the amplitude of OPs induced by mutation of the dopamine D2 receptor, indicating that activation of D2 receptors normally suppresses a light-dependent increase in the amplitudes of OPs. Mutation of the dopamine D2 receptor eliminates the suppression while blocking light stimulation completely diminishes the enhancement of OPs. Taken together with our earlier findings that mutation of the dopamine D1 receptor results in a reduction in OPs amplitude of mice raised under cyclic light conditions to that of dark reared WT mice, and that dark rearing of D1^{-/-} mice has no additional effect on the amplitudes of OPs (He et al., 2013), it strongly suggests a push-pull effect mediated by light through dopamine D1 and D2 receptors on the OPs amplitude. Light stimulation increases dopamine release and the activation of dopamine D1 receptors, which results in an increase of the amplitude of OPs. The dopamine D1 receptor-dependent enhancement of OPs is normally counter balanced by the activation of dopamine D2 receptors, most likely through regulating dopamine release from dopaminergic amacrine cells. Activation of the dopamine D2 receptors on dopaminergic amacrine cells or light deprivation decreases dopamine release and, therefore, decreases the amplitude of OPs by reduced activation of dopamine D1 receptors.

Acknowledgments

This work was supported by A grants R01EY012345, 5P30EY014800 and Research to Prevent Blindness (RPB). We would also like to thank Brent Young and Kevin Huang for their critical and constructive reading and comments to this manuscript.

Abbreviations

ANOCOVA	analysis of covariance
ANOVA	analysis of variance
CNS	central nervous system
ERG	electroretinogram
GABA	γ -aminobutyric acid
LED	Light-emitting diode

NIH	National Institute Health
OPs	oscillatory potentials
RGC	retinal ganglion cell
WT	wild type

References

- Babkoff H. The effect of light deprivation on the B-wave input-output function. *Ann Ophthalmol.* 1975; 7:1335–1338. [PubMed: 1190659]
- Baxter BI, Riesen AH. Electroretinogram of the visually deprived cat. *Science.* 1961; 134:1626–1627. [PubMed: 13866003]
- Ben-Shlomo, Ofri. Development of inner retinal function, evidenced by the pattern electroretinogram, in the rat. *Exp Eye Res.* 2006; 83:417–23. [PubMed: 16626702]
- Bloomfield SA, Völgyi B. The diverse functional roles and regulation of neuronal gap junctions in the retina. *Nat Rev Neurosci.* 2009; 10:495–506. [PubMed: 19491906]
- Bodis-Wollner I, Tzelepi A. The push-pull action of dopamine on spatial tuning of the monkey retina: the effects of dopaminergic deficiency and selective D1 and D2 receptor ligands on the pattern electroretinogram. *Vision Res.* 1998; 38:1479–87. [PubMed: 9667012]
- Braekvelt CR, Hollenberg MJ. The development of the retina of the albino rat. *Am J Anat.* 1970; 127:281–301. [PubMed: 5436824]
- Breton ME, Quinn GE, Schueller AW. Development of electroretinogram and rod phototransduction response in human infants. *Invest Ophthal Vis Sci.* 1995; 36:1588–1602. [PubMed: 7601640]
- Bui BV, Vingrys AJ. Development of receptor responses in pigmented and albino guinea-pigs (*Cavia porcellus*). *Doc Ophthalmol.* 1999; 99:151–170.
- Chergui K. Dopamine induces a GluN2A-dependent form of long-term depression of NMDA synaptic responses in the nucleus accumbens. *Neuropharmacol.* 2011; 60:975–81.
- Derouiche A, Asan E. The dopamine D2 receptor subfamily in rat retina: ultrastructural immunogold and in situ hybridization studies. *Eur J Neurosci.* 1999; 11:1391–402. [PubMed: 10103134]
- DeVries SH, Schwartz EA. Modulation of an electrical synapse between solitary pairs of catfish horizontal cells by dopamine and second messengers. *J Physiol.* 1989; 414:351–375. [PubMed: 2558170]
- Dowling, JE. *The retina.* Cambridge: The Belknap Press of Harvard University Press; 2012.
- Edelmann E, Lessmann V. Dopamine regulates intrinsic excitability thereby gating successful induction of spike timing-dependent plasticity in CA1 of the hippocampus. *Front Neurosci.* 2013; 7:25. [PubMed: 23508132]
- el-Azazi M, Wachtmeister L. The postnatal development of the oscillatory potentials of the electroretinogram. I. Basic characteristics. *Acta Ophthalmol (Copenh).* 1990; 68:401–9. [PubMed: 2220355]
- el Azazi M, Wachtmeister L. The postnatal development of the oscillatory potentials of the electroretinogram. II. Photopic characteristics. *Acta Ophthalmol (Copenh).* 1991a; 69:6–10. [PubMed: 2028769]
- el Azazi M, Wachtmeister L. The postnatal development of the oscillatory potentials of the electroretinogram. III. Scotopic characteristics. *Acta Ophthalmol (Copenh).* 1991b; 69:505–10. [PubMed: 1750319]
- Flores-Guevara R, Renault F, Ostré C, Richard P. Maturation of the electroretinogram in children: stability of the amplitude ratio a/b. *Electroencephalogr Clin Neurophysiol.* 1996; 100:422–7. [PubMed: 8893659]
- Gorfinkel J, Lachapelle P. Maturation of the photopic b-wave and oscillatory potentials of the electroretinogram in the neonatal rabbit. *Can J Ophthalmol.* 1990; 25:138–44. [PubMed: 2361195]

- Gorfinkel J, Lachapelle P, Molotchnikoff S. Maturation of the electroretinogram of the neonatal rabbit. *Doc Ophthalmol*. 1988; 69:237–45. [PubMed: 3168725]
- Hamasaki DI, Maguire GW. Physiological development of the kitten's retina: an ERG study. *Vis Res*. 1985; 25:1537–1543. [PubMed: 3832577]
- He Q, Xu HP, Wang P, Tian N. Dopamine D1 receptors regulate the light dependent development of retinal synaptic responses. *PLoS One*. 2013; 8:e79625. [PubMed: 24260267]
- Hensler JG, Cotterell DJ, Dubocovich ML. Pharmacological and biochemical characterization of the D-1 dopamine receptor mediating acetylcholine release in rabbit retina. *J Pharmacol Exp Ther*. 1987; 243:857–67. [PubMed: 2961878]
- Hensler JG, Dubocovich ML. D1-dopamine receptor activation mediates [3H]acetylcholine release from rabbit retina. *Brain Res*. 1986; 398:407–12. [PubMed: 2948615]
- Herrmann R, Heflin SJ, Hammond T, Lee B, Wang J, Gainetdinov RR, Caron MG, Eggers ED, Frishman LJ, McCall MA, Arshavsky VY. Rod vision is controlled by dopamine-dependent sensitization of rod bipolar cells by GABA. *Neuron*. 2011; 72:101–10. [PubMed: 21982372]
- Herwerth M, Jensen V, Novak M, Konopka W, Hvalby O, Köhr G. D4 dopamine receptors modulate NR2B NMDA receptors and LTP in stratum oriens of hippocampal CA1. *Cereb Cortex*. 2012; 22:1786–98. [PubMed: 21955919]
- Holopigian K, Clewner L, Seiple W, Kupersmith MJ. The effects of dopamine blockade on the human flash electroretinogram. *Doc Ophthalmol*. 1994; 86:1–10. [PubMed: 7956681]
- Huppé-Gourgues F, Coudé G, Lachapelle P, Casanova C. Effects of the intravitreal administration of dopaminergic ligands on the b-wave amplitude of the rabbit electroretinogram. *Vis Res*. 2005; 45:137–45. [PubMed: 15581915]
- Jackson CR, Chaurasia SS, Zhou H, Haque R, Storm DR, Iuvone PM. Essential roles of dopamine D4 receptors and the type 1 adenylyl cyclase in photic control of cyclic AMP in photoreceptor cells. *J Neurochem*. 2009; 109:148–57. [PubMed: 19166506]
- Jackson CR, Ruan GX, Aseem F, Abey J, Gamble K, Stanwood G, Palmiter RD, Iuvone PM, McMahon DG. Retinal dopamine mediates multiple dimensions of light-adapted vision. *J Neurosci*. 2012; 32:9359–68. [PubMed: 22764243]
- Kawai F, Horiguchi M, Miyachi E. Dopamine modulates the voltage response of human rod photoreceptors by inhibiting the h current. *Invest Ophthalmol Vis Sci*. 2011; 52:4113–7. [PubMed: 21421871]
- Kim DY, Jung CS. Gap junction contributions to the goldfish electroretinogram at the photopic illumination level. *Korean J Physiol Pharmacol*. 2012; 16:219–24. [PubMed: 22802705]
- Klitten LL, Rath MF, Coon SL, Kim JS, Klein DC, Møller M. Localization and regulation of dopamine receptor D4 expression in the adult and developing rat retina. *Exp Eye Res*. 2008; 87:471–7. [PubMed: 18778704]
- Kothmann WW, Massey SC, O'Brien J. Dopamine D1-receptor-mediated modulation of connexin36 phosphorylation in AII amacrine cells. *Invest Ophthalmol Vis Sci*. 2008; 49:1515.
- Kothmann WW, Massey SC, O'Brien J. Dopamine-stimulated dephosphorylation of connexin 36 mediates AII amacrine cell uncoupling. *J Neurosci*. 2009; 29:14903–11. [PubMed: 19940186]
- Lankford K, De Mello FG, Klein WL. A transient embryonic dopamine receptor inhibits growth cone motility and neurite outgrowth in a subset of avian retina neurons. *Neurosci Lett*. 1987; 75:169–74. [PubMed: 2952906]
- Lasater EM. Retinal horizontal cell gap junctional conductance is modulated by dopamine through a cyclic AMP-dependent protein kinase. *Proc Natl Acad Sci USA*. 1987; 84:7319–7323. [PubMed: 2823257]
- Lavoie J, Illiano P, Sotnikova TD, Gainetdinov RR, Beaulieu JM, Hébert M. The Electroretinogram as a Biomarker of Central Dopamine and Serotonin: Potential Relevance to Psychiatric Disorders. *Biol Psychiatry*. 2013 pii: S0006–3223(12)01032–3.
- Lorenc-Duda A, Berezi ska M, Urbanska A, Gołembiowska K, Zawilska JB. Dopamine in the Turkey retina-an impact of environmental light, circadian clock, and melatonin. *J Mol Neurosci*. 2009; 38:12–8. [PubMed: 18953673]

- Manglapus MK, Iuvone PM, Underwood H, Pierce ME, Barlow RB. Dopamine mediates circadian rhythms of rod-cone dominance in the Japanese quail retina. *J Neurosci*. 1999; 19:4132–41. [PubMed: 10234041]
- Marmor MF, Fulton AB, Holder GE, Miyake Y, Brigell M, Bach AM. ISCEV Standard for full-field clinical electroretinography (2008 update). *Doc Ophthalmol*. 2009; 118:69–77. [PubMed: 19030905]
- Masland RH. Maturation of function in the developing rabbit retina. *J Comp Neurol*. 1977; 175:275–286. [PubMed: 903424]
- Melamed E, Frucht Y, Vidauri J, Uzzan A, Rosenthal J. Effect of postnatal light deprivation on the ontogenesis of dopamine neurons in rat retina. *Brain Res*. 1986; 391:280–4. [PubMed: 3008950]
- Mills SL, Massey SC. Differential properties of two gap junctional pathways made by AII amacrine cells. *Nature*. 1995; 377:734–737. [PubMed: 7477263]
- Mills SL, Xia XB, Hoshi H, Firth SI, Rice ME, Frishman LJ, Marshak DW. Dopaminergic modulation of tracer coupling in a ganglion-amacrine cell network. *Vis Neurosci*. 2007; 24:593–608. [PubMed: 17711603]
- Miranda-Anaya M, Bartell PA, Menaker M. Circadian rhythm of iguana electroretinogram: the role of dopamine and melatonin. *J Biol Rhythms*. 2002; 17:526–38. [PubMed: 12465886]
- Nguyen-Legros J, Versaux-Botteri C, Vernier P. Dopamine receptor localization in the mammalian retina. *Mol Neurobiol*. 1999; 19:181–204. [PubMed: 10495103]
- Nir I, Harrison JM, Haque R, Low MJ, Grandy DK, Rubinstein M, Iuvone PM. Dysfunctional light-evoked regulation of cAMP in photoreceptors and abnormal retinal adaptation in mice lacking dopamine D4 receptors. *J Neurosci*. 2002; 22:2063–73. [PubMed: 11896146]
- Oliver P, Jolicoeur FB, Lafond G, Drumheller A, Brunette JR. Effects of retinal dopamine depletion on the rabbit electroretinogram. *Doc Ophthalmol*. 1987; 66:359–71. [PubMed: 3123188]
- Popova E, Kупenova P. Effects of dopamine receptor blockade on the intensity- response function of ERG b- and d-waves in dark adapted eyes. *Vision Res*. 2013; 88:22–9. [PubMed: 23810982]
- Perry B, George JS. Dopaminergic modulation and rod contribution in the generation of oscillatory potentials in the tiger salamander retina. *Vision Res*. 2007; 47:309–14. [PubMed: 17184809]
- Reuter JH. The development of the electroretinogram in normal and light-deprived rabbits. *Pflügers Arch*. 1976; 363:7–13. [PubMed: 944912]
- Ribelayga C, Cao Y, Mangel SC. The circadian clock in the retina controls rod-cone coupling. *Neuron*. 2008; 59:790–801. [PubMed: 18786362]
- Ribelayga C, Wang Y, Mangel SC. Dopamine mediates circadian clock regulation of rod and cone input to fish retinal horizontal cells. *J Physiol*. 2002; 544(Pt 3):801–16. [PubMed: 12411525]
- Ribelayga C, Mangel SC. Identification of a circadian clock-controlled neural pathway in the rabbit retina. *PLoS One*. 2010; 5:e11020. [PubMed: 20548772]
- Rodriguez-Saez E, Otero-Costas J, Moreno-Montañes J, Relova JL. Electroretinographic changes during childhood and adolescence. *Eur J Ophthalmol*. 1993; 3:6–12. [PubMed: 8485400]
- Sandalon, Ofri. Age-related changes in the pattern electroretinogram of normal and glatiramer acetate-immunized rats. *Invest Ophthalmol Vis Sci*. 2012; 53:6532–40. [PubMed: 22918635]
- Schneider T, Zrenner E. Effects of D-1 and D-2 dopamine antagonists on ERG and optic nerve response of the cat. *Exp Eye Res*. 1991; 52:425–30. [PubMed: 2037020]
- Severns ML, Johnson MA, Bresnick GH. Methodologic dependence of electroretinogram oscillatory potential amplitudes. *Doc Ophthalmol*. 1994; 86:23–31. [PubMed: 7956683]
- Shelke RR, Lakshmana MK, Ramamohan Y, Raju TR. Levels of dopamine and noradrenaline in the developing of retina--effect of light deprivation. *Int J Dev Neurosci*. 1997; 15:139–43. [PubMed: 9099624]
- Smith WB, Starck SR, Roberts RW, Schuman EM. Dopaminergic stimulation of local protein synthesis enhances surface expression of GluR1 and synaptic transmission in hippocampal neurons. *Neuron*. 2005; 45:765–79. [PubMed: 15748851]
- Spira AW, Parkinson D. Effects of dark-rearing on the retinal dopaminergic system in the neonatal and postnatal guinea pig. *Brain Res Dev Brain Res*. 1991; 62:142–5.

- Stockton RA, Slaughter MM. B-wave of the electroretinogram. A reflection of ON bipolar cell activity. *J Gen Physiol.* 1989; 93:101–122. [PubMed: 2915211]
- Stone RA, Lin T, Iuvone PM, Laties AM. Postnatal control of ocular growth: dopaminergic mechanisms. *Ciba Found Symp.* 1990; 155:45–57. [PubMed: 2088680]
- Sun X, Zhao Y, Wolf ME. Dopamine receptor stimulation modulates AMPA receptor synaptic insertion in prefrontal cortex neurons. *J Neurosci.* 2005; 25:7342–51. [PubMed: 16093384]
- Surmeier DJ, Ding J, Day M, Wang Z, Shen W. D1 and D2 dopamine-receptor modulation of striatal glutamatergic signaling in striatal medium spiny neurons. *Trends Neurosci.* 2007; 30:228–35. [PubMed: 17408758]
- Surmeier DJ, Shen W, Day M, Gertler T, Chan S, Tian X, Plotkin JL. The role of dopamine in modulating the structure and function of striatal circuits. *Prog Brain Res.* 2010; 183:149–67. [PubMed: 20696319]
- Surmeier DJ, Carrillo-Reid L,argas J. Dopaminergic modulation of striatal neurons, circuits, and assemblies. *Neuroscience.* 2011; 198:3–18. [PubMed: 21906660]
- Tian N. Synaptic activity, visual experience and the maturation of retinal synaptic circuitry. *J Physiol.* 2008; 586:4347–4355. [PubMed: 18669531]
- Tian N. Developmental mechanisms that regulate retinal ganglion cell dendrites. *Dev Neurobiol.* 2011; 71:1297–309. [PubMed: 21542137]
- Tian N, Copenhagen DR. Visual stimulation is required for refinement of ON and OFF pathways in postnatal retina. *Neuron.* 2003; 39:85–96. [PubMed: 12848934]
- Tian N, Slaughter MM. Correlation of dynamic responses in the ON bipolar neuron and the b-wave of the electroretinogram. *Vis Res.* 1995; 35:1359–64. [PubMed: 7645264]
- Tucker GS, Hamasaki DI, Labbie A, Bradford N. Physiologic and anatomic development of the photoreceptors of normally-reared and dark-reared rabbits. *Expert Brain Res.* 1982; 48:263–271.
- Urschel S, et al. Protein kinase A-mediated phosphorylation of connexin36 in mouse retina results in decreased gap junctional communication between AII amacrine cells. *J Biol Chem.* 2006; 281:33163–33171. [PubMed: 16956882]
- Van Hook MJ, Wong KY, Berson DM. Dopaminergic modulation of ganglion-cell photoreceptors in rat. *Eur J Neurosci.* 2012; 35:507–18. [PubMed: 22304466]
- Vaquero CF, Pignatelli A, Partida GJ, Ishida AT. A dopamine- and protein kinase A-dependent mechanism for network adaptation in retinal ganglion cells. *J Neurosci.* 2001; 21:8624–35. [PubMed: 11606650]
- Vistamehr S, Tian N. Light deprivation suppresses the light response of inner retina in both young and adult mouse. *Vis Neurosci.* 2004; 21:23–37. [PubMed: 15137579]
- Wachtmeister L. Further studies of the chemical sensitivity of the oscillatory potentials of the electroretinogram (ERG). II. Glutamate-aspartate-and dopamine antagonists. *Acta Ophthalmol (Copenh).* 1981; 59:247–58. [PubMed: 7257744]
- Wachtmeister L. Oscillatory potentials in the retina: what do they reveal. *Prog Retin Eye Res.* 1998; 17:485–521. [PubMed: 9777648]
- Wachtmeister L, Dowling JE. The oscillatory potentials of the mudpuppy retina. *Invest Ophthalmol Vis Sci.* 1978; 17:1176–88. [PubMed: 721390]
- Weber B, Schlicker E, Sokoloff P, Stark H. Identification of the dopamine autoreceptor in the guinea-pig retina as D(2) receptor using novel subtype-selective antagonists. *Br J Pharmacol.* 2001; 133:1243–8. [PubMed: 11498509]
- Weidman TA, Kuwabara T. Postnatal development of the rat retina. An electron microscopic study. *Arch Ophthalmol.* 1968; 79:470–484. [PubMed: 5640327]
- Weidman TA, Kuwabara T. Development of the rat retina. *Invest Ophthalmol.* 1969; 8:60–9. [PubMed: 5763846]
- Westall CA, Panton CM, Levin AV. Time courses for maturation of electroretinogram responses from infancy to adulthood. *Doc Ophthalmol.* 1998–1999; 96:355–79. [PubMed: 10855811]
- Witkovsky P, Stone S, Besharse JC. Dopamine modifies the balance of rod and cone inputs to horizontal cells of the *Xenopus* retina. *Brain Res.* 1988; 449(1–2):332–6. [PubMed: 3293703]

- Wolf ME. Regulation of AMPA receptor trafficking in the nucleus accumbens by dopamine and cocaine. *Neurotox Res.* 2010; 18:393–409. [PubMed: 20361291]
- Xing B, Kong H, Meng X, Wei SG, Xu M, Li SB. Dopamine D1 but not D3 receptor is critical for spatial learning and related signaling in the hippocampus. *Neuroscience.* 2010; 169:1511–9. [PubMed: 20600656]
- Xu HP, Tian N. Pathway specific maturation, visual deprivation and development of retinal pathway. *The Neuroscientist.* 2004; 10:337–346. [PubMed: 15271261]
- Xu TX, Yao WD. D1 and D2 dopamine receptors in separate circuits cooperate to drive associative long-term potentiation in the prefrontal cortex. *Proc Natl Acad Sci U S A.* 2010; 107:16366–71. [PubMed: 20805489]
- Zhang AJ, Jacoby R, Wu SM. Light- and dopamine-regulated receptive field plasticity in primate horizontal cells. *J Comp Neurol.* 2011; 519:2125–34. [PubMed: 21452210]
- Zhu G, Huang Y, Chen Y, Zhuang Y, Behnisch T. MPTP modulates hippocampal synaptic transmission and activity-dependent synaptic plasticity via dopamine receptors. *J Neurochem.* 2012; 122:582–93. [PubMed: 22651101]

Fig. 1. The amplitudes of inner retinal light responses measured as ERG OPs have biphasic changes in D2-/- mice

ERGs were recorded from dark-adapted P30 WT, D2-/- and D3-/- mice at eight different intensities of light stimuli. The amplitudes of a-wave, b-wave and OPs were plotted as a function of intensity of light stimuli as intensity-response curve and the amplitudes of ERG a-, b-wave and OPs of D2-/- mice were also normalized to the WT controls to reveal the relative changes of strength of ERG of D2-/- mice. **A:** Representative ERG (left) and OPs (right) waveforms recorded from a P30 WT mouse (upper) and a P30 D2-/- mouse (lower) evoked by 8 different light intensities (from 0.008 cd*s/m² at the bottom to 25 cd*s/m² at the top). **B:** Average intensity-response curves of a-wave amplitude of WT (20 mice, 40 eyes), D2-/- (7 mice, 14 eyes) and D3-/- (10 mice, 20 eyes) mice. **C:** Normalized ERG a-wave of WT and D2-/- mice shows that the a-wave amplitudes of D2-/- mice were not different from that of WT mice at all light intensities except the lowest intensity of light stimulus (0.008 cd*s/m²). The amplitude of a-wave of D2-/- mice evoked by 0.008 cd*s/m² is more than 2-fold higher than that of WT controls. **D:** Average intensity-response curves of b-wave amplitude of the same three groups of mice as shown in Fig 1B. **E:** Normalized ERG b-wave of WT and D2-/- mice shows that the b-wave amplitudes of D2-/- mice were not different from that of WT mice at all light intensities except the lowest intensity of light stimulus. The amplitude of b-wave of D2-/- mice evoked by 0.008 cd*s/m² is about 30% lower than that of WT controls. **F:** Average intensity-response curves of OPs amplitude of the same three groups of mice as shown in Fig 1B. **G:** Normalized ERG OPs of WT and D2-/- mice shows that the OPs amplitudes of D2-/- mice have biphasic changes, reduced to low light intensity stimuli while enhanced to high light intensity stimuli. At the low light intensity (0.025 cd*s/m²), the amplitude of OPs of D2-/- mice is about 30% lower than that of WT controls, while at the high light intensities (2.5–25 cd*s/m²) the amplitudes of OPs of D2-/- mice are about 30–35% higher than that of WT controls. In all panels, * indicates the difference is statistically significant and p value is between 0.05 and 0.01; ** indicates p value is smaller than 0.01; error bars indicate standard errors in this and all following figures.

Fig 2. The response gains of inner retina to low and high intensity of stimuli are differentially affected in D2-/- mice

The ratio of b/a-wave was used to assess the response gain of outer retina and the ratio of OPs/b-wave was used to assess the response gain of inner retina. The response gains of D2-/- mice were normalized to that of WT controls to reveal the relative changes of the response gains of D2-/- mice. **A:** The b/a-wave ratios of WT and D2-/- mice plotted as a function of the light intensity showing a slight to moderate increase of the response gains at the light intensities of 0.025–0.25 cd*s/m². **B:** The b/a-wave ratios of D2-/- mice normalized to WT controls. **C:** The OPs/b-wave ratios of WT and D2-/- mice plotted as a function of the light intensity showing a significant light intensity-dependent increase of the response gain for both WT and D2-/- mice. **D:** The OPs/b-wave ratios of D2-/- mice normalized to WT controls showing similar light intensity-dependent biphasic changes, 20% decrease of the response gain at the light intensity of 0.025 cd*s/m² but 25–30% increase at the light

intensities of 2.5–25 cd*s/m². **E:** The time to peak of a-wave of WT and D2^{-/-} mice plotted as a function of the light intensity showing minimum difference between WT and D2^{-/-} mice. **F:** The time to peak of b-wave of WT and D2^{-/-} mice plotted as a function of the light intensity showing minimum difference between WT and D2^{-/-} mice. The data was from the same groups of WT and D2^{-/-} mice as shown in Fig 1.

Fig 3. Dopamine D2 receptor preferentially regulates the development of bipolar and inner retina light responsiveness evoked by low intensity of lights after eye opening
 ERGs were recorded from dark-adapted WT and D2^{-/-} mice at the age of P13. The amplitudes of a-wave, b-wave and OPs were plotted as a function of intensity of light stimuli and the amplitudes of ERG a-wave, b-wave and OPs of P13 WT and D2^{-/-} mice were compared with that of P30 mice to reveal the developmental changes of ERG amplitudes. **A:** Average intensity-response curves of a-wave amplitude of P13 WT (n = 12 eyes of 6 mice) and D2^{-/-} (n = 18 eyes of 9 mice) mice. **B:** The P30/P13 ratios of ERG a-wave amplitudes of WT and D2^{-/-} mice as a function of light intensity. **C:** Average intensity-response curves of b-wave amplitude of the same groups WT and D2^{-/-} mice as for panel A. **D:** The P30/P13 ratios of b-wave amplitudes of WT and D2^{-/-} mice as a function of light intensity, showing selective reduction of the P30/P13 ratios for low intensity of lights. **E:** Average intensity-response curves of OPs of the same groups WT and

D2^{-/-} mice as for panel A. **F:** The P30/P13 ratios of OPs amplitudes of WT and D2^{-/-} mice as a function of light intensity, also showing selective reduction of the P30/P13 ratios for low intensity of lights.

Fig 4. Light deprivation suppresses the ERG amplitudes of both WT and D2^{-/-} mice

To determine the effects of light deprivation on ERG amplitudes, WT and D2^{-/-} mice were raised in constant darkness from birth and the ERGs were recorded at P30. **A:** Average intensity-response curves of a-wave amplitude of WT mice raised in cyclic light/dark conditions (WT Light, 20 mice, 40 eyes) and constant darkness (WT Dark, 7 mice, 14 eyes). **B:** Average intensity-response curves of b-wave amplitude of WT mice raised in cyclic light/dark conditions and constant darkness. **C:** Average intensity-response curves of OPs of WT mice raised in cyclic light/dark conditions and constant darkness showing significant decrease of the OPs amplitudes of dark-reared mice. **D:** Average intensity-response curves of OPs amplitude of WT and D2^{-/-} mice raised under cyclic light/dark conditions, showing significant increase of the OPs amplitudes evoked by high intensity of lights of D2^{-/-} mice. **E:** Average intensity-response curves of a-wave amplitude of D2^{-/-} mice raised in cyclic light/dark conditions (D2^{-/-} Light, 7 mice, 14 eyes) and constant darkness (D2^{-/-} Dark, 9 mice, 18 eyes). **F:** Average intensity-response curves of b-wave amplitude of D2^{-/-} mice raised in cyclic light/dark conditions and constant darkness. **G:** Average intensity-response curves of OPs of D2^{-/-} mice raised in cyclic light/dark conditions and constant darkness showing significant decrease of the OPs amplitudes, especially the responses to high intensity of lights, of D2^{-/-} mice raised in constant darkness. **H:** Average intensity-response curves of OPs amplitude of WT and D2^{-/-} mice raised in constant darkness, showing that light deprivation suppressed the OPs amplitude of WT and D2^{-/-} mice to the same level.

Fig 5. Dopamine D2 receptors mediate the light-sensitive response gain of both outer and inner retina

The b/a-wave ratios and OPs/b-waves ratios of WT and D2^{-/-} mice raised under cyclic light/dark conditions and constant darkness were used to assess the effects of light deprivation on the response gains of ERG. **A:** The b/a-wave ratios of WT mice raised under cyclic light/dark conditions and constant darkness were plotted as a function of the light intensity showing a significant increase of the outer retina response gain at the light intensities of 0.008–0.08 cd*s/m² of dark reared WT mice. **B:** The OPs/b-wave ratios of WT mice raised under cyclic light/dark conditions and constant darkness. **C:** The b/a-wave ratios of D2^{-/-} mice raised under cyclic light/dark conditions and constant darkness showing no

significant change of the outer retina response gain in dark reared D2^{-/-} mice. **D:** The OPs/b-wave ratios of D2^{-/-} mice raised under cyclic light/dark conditions and constant darkness showing a significant decrease of the inner retina response gain selective to responses evoked by high intensity of lights. **E:** The b/a-wave ratios of D2^{-/-} mice raised in constant dark were normalized to D2^{-/-} mice raised in cyclic light/dark conditions while the b/a-wave ratios of WT mice raised in constant dark were normalized to WT mice raised in cyclic light/dark conditions. These normalized b/a-wave ratios were plotted as functions of light intensities and showed that light deprivation significantly increased the b/a-wave ratios of light responses evoked by low intensity of lights in WT but not D2^{-/-} mice. **F:** The OPs/b-wave ratios of D2^{-/-} and WT mice raised in constant dark were normalized to control mice raised in cyclic light/dark conditions, respectively, and plotted as functions of light intensities.

Fig 6. Mutation of dopamine D2 receptor diminishes the effects of light deprivation on the kinetics of ERG

The time to peak of ERG a-wave and b-wave of WT and D2^{-/-} mice raised under cyclic light/dark conditions and constant darkness were measured and plotted as a function of the light intensity. **A:** The time to peak of a-wave of WT mice raised under cyclic light/dark conditions and constant darkness plotted as a function of the light intensity. **B:** The time to peak of b-wave of WT mice raised under cyclic light/dark conditions and constant darkness plotted as a function of the light intensity. **C:** The time to peak of a-wave of D2^{-/-} mice raised under cyclic light/dark conditions and constant darkness plotted as a function of the

light intensity. **D:** The time to peak of b-wave of D2^{-/-} mice raised under cyclic light/dark conditions and constant darkness plotted as a function of the light intensity. **E:** The time to peak of ERG a-wave of D2^{-/-} and WT mice raised in constant dark were normalized to that of D2^{-/-} and WT control mice raised in cyclic light/dark conditions, respectively, and plotted as functions of light intensities, showing that the effects of light deprivation on the time to peak of ERG a-wave of WT mice were diminished in D2^{-/-} mice. **F:** The time to peak of ERG b-wave of D2^{-/-} and WT mice raised in constant dark were normalized to control mice raised in cyclic light/dark conditions, respectively, and plotted as functions of light intensities.

Table 1

Predicted maximal ERG responses

	P13						P30					
	a-wave		b-wave		OPs		a-wave		b-wave		OPs	
	WT	D2-/-	WT	D2-/-	WT	D2-/-	WT	D2-/-	WT	D2-/-	WT	D2-/-
Mean (μV)	-53.5	-47.7	230.4	332.7	99	160.8	-445.2	-446.9	1108.1	1046.3	914.3	1076.4
SE (μV)	8.4	10.3	43.7	41.9	14.6	22.1	18	37.4	44	75.9	44.4	73.9
n (eyes)	12	15*	12	18	12	18	38*	11*	40	14	39*	14
p	<0.0001		0.1133		0.0462		0.964		0.4804		0.0664	
t	-7.334		1.635		2.086		-0.045		-0.711		1.878	