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Age-related changes in visual function in *cystathionine-beta-synthase* mutant mice, a model of hyperhomocysteinemia

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Abstract

Homocysteine is an amino acid required for the metabolism of methionine. Excess homocysteine is implicated in cardiovascular and neurological disease and new data suggest a role in various retinopathies. Mice lacking *cystathionine-beta-synthase* (*cbs*^{-/-}) have an excess of retinal homocysteine and develop anatomical abnormalities in multiple retinal layers, including photoreceptors and ganglion cells; heterozygous (*cbs*^{+/-}) mice demonstrate ganglion cell loss and mitochondrial abnormalities in the optic nerve. The purpose of the present study was to determine whether elevated homocysteine, due to absent or diminished *cbs*, alters visual function. We examined *cbs*^{-/-} (3 weeks) and *cbs*^{+/-} mice (5, 10, 15, 30 weeks) and results were compared to those obtained from wildtype (WT) littermates. Light- and dark-adapted ERGs were recorded along with dc-ERG to assess retinal pigment epithelial (RPE) function. The visual evoked potential (VEP) was used to assess transmission to the visual cortex. The amplitudes of the major ERG components were reduced in *cbs*^{-/-} mice at age 3 weeks and VEPs were delayed markedly. These findings are consistent with the early retinal disruption observed anatomically in these mice. In comparison, at 3 weeks of age, responses of *cbs*^{+/-} mice did not differ significantly from those of WT mice. Functional abnormalities were not observed in *cbs*^{+/-} mice until 15 weeks of age, at which time amplitude reductions were noted for the ERG a- and b-wave and the light peak component, but not for other components generated by the RPE. VEP implicit times were delayed in *cbs*^{+/-} mice at 15 and 30 weeks. The later onset of functional defects in *cbs*^{+/-} mice is consistent with a slow loss of ganglion cells reported previously in the heterozygous mutant. Light peak abnormalities indicate that RPE function is also compromised in older *cbs*^{+/-} mice. The data suggest that severe elevations of homocysteine are associated with marked alterations of retinal function while modest homocysteine elevation is reflected in modest and delayed alterations of retinal function. The work lays the foundation to explore the role of homocysteine in retinal diseases such as glaucoma and optic neuropathy.

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Keywords

electroretinogram; visual evoked potential; retina; optic nerve; mouse; retinal function; electrophysiology; retinal degeneration

1. Introduction

Homocysteine, a nonproteinogenic and sulfur-containing amino acid, is an intermediate compound in methionine metabolism. Excess homocysteine is detrimental to normal physiological function; hence homocysteine is typically remethylated to methionine or converted via the transsulfuration pathway to cysteine under the control of the enzyme cystathionine-beta-synthase (CBS). CBS activity plays a critical role in regulating homocysteine concentrations, and CBS dysfunction is associated with elevated homocysteine levels. In human, *CBS* mutations underlie a complex phenotype with ocular, skeletal, neurological and cardiovascular components (Mudd, 2011).

In patient-based studies, elevated homocysteine has been detected in a number of clinical conditions affecting the eye, including AMD (Axer-Siegel *et al.*, 2004; Seddon *et al.*, 2006); maculopathy and retinal degeneration (Tsina *et al.*, 2005); primary and secondary open-angle glaucoma (Bleich *et al.*, 2002); exfoliative glaucoma (Vessani *et al.*, 2003), pigmentary glaucoma (Jaksic *et al.*, 2010), proliferative vitreoretinopathy (Aydemir *et al.*, 2008), type 2 diabetes mellitus (Yang *et al.*, 2002), central retinal vein occlusion (Lahey *et al.*, 2003). Poloschek *et al.* (2005) reported abnormal electroretinograms (ERGs) and visual evoked potentials (VEPs) in a child with severe hyperhomocysteinemia due to methionine synthase deficiency.

To understand mechanisms of homocysteine-induced disruption of retina, *in vitro* and *in vivo* model systems have been used. Homocysteine upregulates VEGF expression and induces ER stress in cultured ARPE-19 cells (Roybal *et al.*, 2004). It induces apoptotic death in the RGC-5 cell line (Ganapathy *et al.*, 2010) and very modest levels of homocysteine induce apoptotic death of primary ganglion cells harvested from neonatal mice (Dun *et al.*, 2007). Analysis of retinas of mice in which high concentrations of homocysteine were administered via an intravitreal route revealed marked ganglion cell loss and disruption of the inner retina within a few days of injection (Moore *et al.*, 2001) and significant loss of photoreceptor cells when the retinas were exposed to homocysteine for several months (Chang *et al.*, 2011). The availability of a mouse in which the *cbs* gene is deleted (*cbs*^{-/-}) (Watanabe *et al.*, 1995) has permitted analysis of the effects of endogenous elevation of homocysteine on retinal neurons. Depending upon whether the mouse has one *cbs* allele (*cbs*^{+/-}) or no copies of *cbs* (*cbs*^{-/-}), the plasma homocysteine ranges from moderate to severe, respectively. Comprehensive morphological analysis of retinas of homozygous mutant mice (*cbs*^{-/-}) revealed alterations in the RPE, loss of cells in the ganglion cell layer, and marked disruption of the inner/outer nuclear retinal layers (Ganapathy *et al.*, 2009). These mice have ~30-fold increase in plasma homocysteine, ~7-fold increase in retinal homocysteine and a lifespan of ~3–5 weeks; they are a model of severe hyperhomocysteinemia (Watanabe *et al.*, 1995). Analysis of retinas of heterozygous mice (*cbs*^{+/-}) reveals a much milder phenotype characterized by modest cell loss in the ganglion cell layer and decreased thickness of the inner plexiform and nuclear layers. Gross disruption of the retina has not been observed in heterozygous mice (*cbs*^{+/-}), which have ~4–7-fold increase in plasma homocysteine and reflect moderate hyperhomocysteinemia. Interestingly, the retinal ganglion cell loss is accompanied by alterations of the mitochondria of the nerve fiber layer detectable at the ultrastructural level and by increased expression of two mitochondrial proteins, Opa1 and Fis1 (Ganapathy *et al.*, 2011). Thus, the histological

appearance of retinas of the *cbs* mutant mice has been analyzed comprehensively; however it is not known what functional alterations accompany these retinal abnormalities. The present study addressed this by using visual electrophysiological protocols to study *cbs* mutant mice at different ages.

2. Methods

2.1. Mice

Generation of mice deficient in *cbs* was reported by Watanabe *et al.* (1995) and a colony of mice has been established at the Jackson Laboratories (Bar Harbor, ME). Breeding pairs of *cbs*^{+/-} mice (B6.129P2-Cbs^{tm1Unc/J}) were used to generate *cbs*^{-/-}, *cbs*^{+/-} and *cbs*^{+/+} mice. Genotyping, husbandry and housing conditions for the mice were as described (Ganapathy *et al.*, 2009). All experiments involving mice were approved by the Institutional Animal Care and Use Committee of the Cleveland Clinic Foundation and adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

2.2. Visual Electrophysiology

Three recording protocols were used. All were conducted following overnight dark adaptation, after which mice were anesthetized with ketamine (80 mg/kg) and xylazine (16 mg/kg), and placed on a temperature-regulated heating pad. The pupils were dilated with eyedrops (2.5% phenylephrine HCl, 1% cyclopentolate, 1% tropicamide); for ERG studies, the corneal surface was anesthetized with 1% proparacaine HCL eyedrops.

2.2.1. Dark- and light-adapted ERG—To evaluate responses of the outer retina, we used a conventional strobe-flash ERG protocol. The ERG was recorded using a stainless-steel wire active electrode that contacted the corneal surface through 1% methylcellulose. Needle electrodes placed in the cheek and tail served as reference and ground leads, respectively. Responses were differentially amplified (0.3–1,500 Hz), averaged, and stored using a UTAS E-3000 signal averaging system (LKC Technologies, Gaithersburg, MD). Strobe flash stimuli were initially presented in darkness within a ganzfeld bowl; across-trials flash luminance ranged from 3.6 to 2.1 log cd s/m². Stimuli were presented in order of increasing luminance and the interstimulus interval (ISI) was progressively increased from 4 to 90 s. A steady adapting field (20 cd/m²) was presented in the ganzfeld. After a 7 min light adaptation period cone ERGs were evoked by strobe flash stimuli superimposed upon the adapting field. Flash luminance ranged from -0.8 to 1.9 log cd s/m². Stimuli were presented at 2 Hz in order of increasing luminance and 25 successive responses were averaged for each stimulus condition. The amplitude of the a-wave was measured 7 ms after flash onset from the prestimulus baseline. The amplitude of the b-wave was measured from the a-wave trough to the peak of the b-wave or, if no a-wave was present, from the prestimulus baseline.

2.2.2. Direct current (dc)-ERG—To measure ERG components generated by the RPE, responses were obtained from the corneal surface of the left eye using a 1-mm-diameter borosilicate capillary tube with filament (BF100-50-10; Sutter Instrument, Novato, CA). The capillary was filled with Hank's buffered salt solution to make contact with an Ag/Ag Cl wire electrode that was shielded from light stimulation. A similar electrode placed in contact with the right eye served as the reference. Responses were differentially amplified at dc-100 Hz (gain = ×1,000; DP-301, Warner Instruments, Hamden, CT), digitized at 20 Hz, and stored using LabScribe Data Recording Software (iWorx; Dover, NH). After the initial setup for each mouse was complete, the recorded baseline was monitored until the baseline became stable. White light stimuli were then derived from an optical channel using a Leica microscope illuminator as the light source and delivered to the test eye with a 1-cm-diameter

fiber-optic bundle. The stimulus luminance was 2.4 log cd/m². A Uniblitz shutter system was used to control stimulus duration at 7 min.

The mouse dc-ERG has four major components (Wu *et al.*, 2004). The amplitude of the c-wave was measured from the prestimulus baseline to the peak of the c-wave. The amplitude of the fast oscillation (FO) was measured from the c-wave peak to the trough of the FO. The amplitude of the light peak (LP) was measured from the FO trough to the asymptotic value. The off-response amplitude was measured from the LP value just prior to stimulus light offset to the peak of the initial component.

2.2.3. Visual evoked potential—To measure responses of the visual cortex, VEPs were recorded using an active electrode positioned along the midline of the visual cortex. The positions of the reference and ground leads were the same as for ERG recordings and the bandpass filter settings were 1–100 Hz. VEPs were recorded to achromatic strobe flash stimuli presented in the LKC ganzfeld under dark-adapted conditions. The ISI ranged from 1.1 to 6 s, increasing with stimulus intensity from –2.4 to 2.1 log cd s/m²; 60 to 20 successive responses were averaged to obtain a VEP waveform. The mouse VEP is dominated by a negative component which is referred to as N1 (Peachey *et al.*, 1997; Ridder and Nusinowitz, 2006; Yu *et al.*, 2011). The implicit time of the N1 component was measured at the negative peak.

2.3. Statistical analysis

Response measures were analyzed statistically using repeated measures ANOVA. Because the standard error was not constant across all pairs of means, the degrees of freedom were adjusted by multiplying by the degrees of freedom of the Greenhouse-Geisser epsilon, and significance was adjusted accordingly. Where only a single stimulus intensity was used, data were analyzed using group Student's t-test.

2.4. Histology

Comprehensive morphometric analyses of retinas of *cbs*^{–/–}, *+/-*, and *+/+* mice have been reported (Ganapathy *et al.*, 2009). To confirm these observations and relate the electrophysiological data to retinal histology, eyes were immersion fixed in a solution of 2% paraformaldehyde/2.5% glutaraldehyde in 0.1 M cacodylate buffer and prepared for embedding in JB-4 solution ((Electron Microscopy Sciences (EMS), Hatfield, PA) per our method (Ha *et al.*, 2011). Retinal images were captured using an Axioplan-2 microscope equipped with an HRM camera (Carl Zeiss, Oberkochen, Germany).

3. Results

3.1. Dark- and light-adapted ERG: correlation with retinal histology

Figure 1 presents ERG data obtained from 3-week old *cbs*^{+/+} (WT), *cbs*^{+/-} and *cbs*^{–/–} mice. At this age, dark-adapted responses of WT and *cbs*^{+/-} mice were comparable in waveform and amplitude, while those of *cbs*^{–/–} mice were significantly reduced in amplitude (Fig. 1A, B). A similar result was obtained for the light-adapted ERG as the responses of WT and *cbs*^{+/-} mice were very similar while those of *cbs*^{–/–} mice were significantly reduced in amplitude (Fig. 1C, D). The normal ERG observed in *cbs*^{+/-} mice at this age is consistent with the normal retinal histology observed in wildtype mice (Fig. 2A, B) and *cbs*^{+/-} mice (data not shown). In the case of the *cbs*^{–/–} mice, the altered electrophysiologic response (Fig. 1) is reflected in the disrupted morphologic appearance of the *cbs*^{–/–} retina (Fig 2C–F). The retinal appearance in the homozygous mutant mice is variable, with some areas having a typical laminar appearance and only mild alterations (Fig. 2C,D) and other areas where the laminar appearance is altered significantly marked by areas of cellular hypertrophy (Fig. 2E)

and rosette formation within the nuclear layers (Fig. 2F, arrows). These morphologic findings are similar to our previous report indicating that *cbs*^{-/-} retinas have regions of profound ganglion cell dropout and disruption of the inner and outer nuclear layers (Ganapathy *et al.*, 2009).

Owing to the shortened lifespan of *cbs*^{-/-} mice of (~3 weeks) (Watanabe *et al.*, 1995), it was not feasible to obtain ERG data from older *cbs*^{-/-} mice. Consequently, we concentrated much of our functional analyses on *cbs*^{+/-} mice in which a slow progressive loss of retinal cells has been reported (Ganapathy *et al.*, 2009). Figure 3 compares dark-adapted (A–D) and light-adapted (E–H) intensity-response functions for WT and *cbs*^{+/-} littermates at 5 weeks (Fig. 3A, E), 10 weeks (Fig. 3B, F), 15 weeks (Fig. 3C, G) and 30 weeks of age (Fig. 3D, H). Responses of *cbs*^{+/-} mice did not differ from WT at 5 or 10 weeks of age. By 15 weeks of age, however, *cbs*^{+/-} responses were significantly reduced in amplitude, under both dark- and light-adapted conditions (Fig. 3C,G). A similar reduction in amplitude was noted at the oldest age examined, 30 weeks (Fig. 3D,H).

Hematoxylin and eosin-stained retinal sections of 30 week old WT and *cbs*^{+/-} mice are shown in Figure 4. The retinas of *cbs*^{+/-} mice are generally well-organized and the nuclear and plexiform layers are similar in appearance and thickness to *cbs*^{+/+} mice. Figure 4A and B provide low power photomicrographs of retinas of the WT and *cbs*^{+/-} mice, respectively. Higher magnification photomicrographs of the inner retina, middle retina and outer retina are shown in Fig. 4C and D, 4E and F, 4G and H, respectively. Aside from the thinner inner plexiform layer and ganglion cell drop-out, the histological appearance of the inner and outer nuclear layers is well-organized.

3.2. RPE Electrophysiology

We used dc-ERG recordings to evaluate RPE function in WT and *cbs*^{+/-} mice. Figure 5 compares average dc-ERGs obtained from mice at 5 weeks (A) and 30 weeks (B) of age. At 5 weeks, there was no difference between WT and *cbs*^{+/-} mice in any of the major components of dc-ERG (c-wave, fast oscillation, light peak, off-response). At 30 weeks, the light peak component was significantly reduced in *cbs*^{+/-} mice, while all other response components were not different from WT. The light peak reduction was not accompanied by marked alterations in the histologic appearance of the RPE (data not shown).

3.3. Visual Evoked Potentials

To evaluate how the ganglion cell loss reported in *cbs*^{+/-} mice (Ganapathy *et al.*, 2009) impacts visual transmission to the visual cortex, we recorded VEPs to strobe flash stimuli presented in the dark. Figure 6A shows representative VEP waveforms obtained using 3 stimulus intensities from a WT mouse and a *cbs*^{+/-} littermate at 30 weeks of age. The mouse VEP is dominated by a large negative wave (N1), the implicit time of which decreases with increasing flash intensity (Yu *et al.*, 2011). Intensity-response functions for N1 implicit time are shown in Figure 6B–D for mice tested at 5 weeks (B), 10 weeks (C) or 30 weeks (D) of age. At 5 and 10 weeks of age, there was no difference in N1 implicit time between WT and *cbs*^{+/-} mice. At 30 weeks of age, however, N1 implicit time was delayed significantly in *cbs*^{+/-} mice.

Given the marked disruption of the *cbs*^{-/-} retina (Ganapathy *et al.*, 2009), we also recorded the VEP from these animals, albeit only at 3 weeks of age owing to their premature death. Figure 7 shows that N1 implicit time was markedly delayed in *cbs*^{-/-} mice as compared with age-matched heterozygous and wildtype littermates. These data indicate altered optic nerve function at this very early age in mice with extremely high levels of homocysteine.

4. Discussion

The present study represents the first comprehensive analysis of retinal function in an animal model of hyperhomocysteinemia due to deficiency of CBS. In mice with no CBS protein (*cbs*^{-/-}) the electrophysiologic responses of the retina were markedly diminished at an early age. The dark- and light-adapted a- and b-waves, though discernable, were significantly reduced compared to WT and *cbs*^{+/-} littermates. The *cbs*^{-/-} mice also manifest abnormal retinal output to the visual cortex, as shown by the marked delay in VEP implicit time. Although additional defects may be present in the central visual pathway, these electrophysiological data are consistent with the pathologic changes observed in *cbs*^{-/-} retinas, including disruption of the architecture of the inner and outer nuclear layers (Ganapathy *et al.*, 2009; Fig. 2). In humans with severe deficiency of CBS, the retina has not been examined by microscopy or with imaging modalities. The first sign of ocular disturbance is typically dislocation of the lens due to degenerative changes in the zonular fibers that suspend the lens from the ciliary body (Mulvihill *et al.*, 2001). Glaucoma occurs in most patients as an acute and painful event following lens dislocation (reviewed by Gerding, 1998).

In *cbs*^{+/-} mice with only a modest increase in plasma homocysteine there was a gradual loss of retinal electrophysiologic function. At early ages (5, 10 weeks) the light- and dark-adapted a- and b-wave amplitudes were similar to age-matched WT animals reflecting normal function of photoreceptor cells and bipolar cells (Frishman and Wang, 2011). Functional changes were observed in older mice (15, 30 weeks), ages when retinal alterations begin to be appreciated (Ganapathy *et al.*, 2009). The reduced dc-ERG light peak was not accompanied by other electrophysiological manifestations of RPE dysfunction. Component-specific changes have been noted in dc-ERG studies of other mouse mutants (Wu *et al.*, 2007; Edwards *et al.*, 2010).

VEP recordings revealed marked differences in visual transmission to the visual cortex in 30-week old *cbs*^{+/-} mice compared to WT littermates. These VEP delays are noteworthy given the earlier documentation of ganglion cell loss in *cbs*^{+/-} mice (Ganapathy *et al.*, 2009) and the recent report that mitochondrial dynamics are altered in retinal ganglion cell axons of *cbs* mutant mice (Ganapathy *et al.*, 2011). Ganapathy and colleagues noted that in comparison to WT, the *cbs*^{+/-} retina has elevated levels of two mitochondrial proteins, Opa1 and Fis1, and that mitochondrial abnormalities could be observed ultrastructurally. Alterations in Opa1 are associated with glaucoma and optic neuropathies and mitochondrial dysfunction is implicated in the pathogenesis of these diseases (Carelli *et al.*, 2004). Our functional evidence demonstrating delayed transmission to the visual cortex provides additional support for a role of elevated homocysteine in the pathogenesis of glaucoma. These data correlate with the ganglion cell losses noted here in *cbs*^{+/-} mice and reported earlier (Ganapathy *et al.*, 2009; Ganapathy *et al.*, 2011). Within the past few years there have been several clinical studies reporting elevated homocysteine associated with various forms of glaucoma (Tranchina *et al.*, 2011; Turgut *et al.*, 2010; Jaksic *et al.*, 2010; Micheal *et al.*, 2009; Clement *et al.*, 2009). It will be informative to determine whether mutations of *CBS* and genes encoding other proteins involved in homocysteine metabolism contribute to some forms of glaucoma or optic nerve dysfunction.

Severe hyperhomocysteinemia can be caused by mutations in genes other than *cbs*. For example, elevations in plasma homocysteine are caused by mutations in *methylenetetrahydrofolate reductase (MTHFR)*, an important enzyme in homocysteine metabolism that catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the predominant circulating form of folate. ERG studies of *mthfr*^{-/-} mice have been recently reported (Lawrance *et al.*, 2011). Both dark- and light-adapted

ERGs of 6 week *methfr*^{-/-} mice were smaller than WT controls. This reduction was not progressive, however, and no difference was noted when mice were tested at 13 weeks of age. Although VEPs were not recorded, it is important to note that several cases of *MTHFR* mutations and visual defects associated with diabetic retinopathy, anterior ischemic optic neuropathy and glaucoma have been reported (Maeda *et al.*, 2006; Felekis *et al.*, 2010; Jaksic *et al.*, 2010).

Methionine synthase is an enzyme, encoded by the *MTR* gene (5-methyltetrahydrofolate-homocysteine methyltransferase), which remethylates homocysteine to methionine using 5-methyltetrahydrofolate. Mutations in *MTR* are associated with elevated homocysteine and retinal abnormalities. Poloschek *et al.* (2005) performed comprehensive retinal electrophysiological evaluation over several years in a young patient with methionine synthase deficiency and reported decreased amplitude of the a- and b-waves consistent with decreased photoreceptor function. In this patient, the flash VEP waveform was severely distorted. While this precluded precise implicit time measurements, this result is consistent with ganglion cell loss. Interestingly, reduced oscillatory potentials were also observed in this patient that may reflect microvascular damage in the inner retina.

Taken collectively, the data suggest that marked elevation of homocysteine has a deleterious effect on retinal function and modest elevation can alter retinal function and the retinal output signal. Determining the mechanism by which homocysteine alters retinal function, particularly that of ganglion cells under moderate homocysteine conditions and other retinal neurons under severe hyperhomocysteinemic conditions will be the focus of future work. The availability of *cbs* and *methfr* mutant mice, with endogenous elevation of homocysteine, offers excellent experimental tools with which to pursue this line of inquiry.

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Figure 1.

ERG results obtained from 3 week old mice. (A) ERGs obtained from WT (*cbs*^{+/+}), *cbs*^{+/-}, and *cbs*^{-/-} mice to strobe flash stimuli presented to the dark-adapted eye. (B) Intensity-response functions for the major ERG components. Responses of WT and *cbs*^{+/-} mice are not statistically different. Responses of *cbs*^{-/-} mice are significantly reduced in amplitude (a-wave: $p < 0.02$; b-wave: $p < 0.005$). (C) Cone ERGs obtained to strobe flash stimuli superimposed upon a steady adapting field. (D) Intensity responses for the cone ERG. In comparison to WT, cone ERGs of *cbs*^{+/-} mice are comparable in amplitude while *cbs*^{-/-} mice are significantly reduced in amplitude ($p < 0.05$). Scale bar in A indicates 200 μV and 100 ms. Scale bar in C indicates 100 μV and 100 ms. In B and D, data points indicate the average ($\pm$ SD) of 19 WT, 26 *cbs*^{+/-} and 4 *cbs*^{-/-} mice.

Figure 2.

Histology of retinas of 3 week old mice. Light micrographs of hematoxylin and eosin-stained inner (A) and outer (B) retinal regions in WT (*cbs*^{+/+}) mice; inner (C) and outer (D) regions of homozygous mutant (*cbs*^{-/-}) mice. Photomicrographs in E and F provide lower magnification of retinas of *cbs*^{-/-} mice showing altered retinal architecture including rosette formation (arrows) within the nuclear layers. Calibration bar = 50 μ m. Abbreviations: gcl = ganglion cell layer, ipl = inner plexiform layer, inl = inner nuclear layer, onl = outer nuclear layer, rpe = retinal pigment epithelium.

Figure 3.

ERG changes in *cbs*^{+/-} heterozygous mice. ERG intensity-response functions for WT and *cbs*^{+/-} mice at 5 weeks (A, E), 10 weeks (B, F), 15 weeks (C, G) and 30 weeks (D, H) of age were obtained under dark-adapted (A–D) and light-adapted (E–H) conditions. For the dark-adapted condition, there was no significant difference between WT and *cbs*^{+/-} mice at 5 or 10 weeks of age. At 15 and 30 weeks of age, both a- and b-waves were significantly reduced in *cbs*^{+/-} mice ($p < 0.03$). Light-adapted responses did not differ between WT and *cbs*^{+/-} mice at 5 or 10 weeks of age, but *cbs*^{+/-} responses were significantly reduced at 15 and 30 weeks of age ($p < 0.03$ and 0.05 , respectively). Data points indicate average ($\pm$ SD) of 4–12 different mice.

Figure 4.

Histology of retinas of 30 week old heterozygous and wildtype mice. Low magnification light micrographs of hematoxylin and eosin-stained retinas from (A) WT (*cbs*^{+/+}) and (B) heterozygous (*cbs*^{+/-}) mice. Higher magnification light photomicrograph images of inner (C,D), middle (E,F) and outer (G,H) retina of WT and *cbs*^{+/-} mice, respectively. Generally, the laminar organization of the retina in *cbs*^{+/-} mice is similar to that of WT mice, although the inner plexiform layer is slightly reduced in thickness and there is clear evidence of dropout of ganglion cells (arrows in panel B) in these retinas. There are occasional aberrations observed in the region of the RPE as shown in panel H. Calibration bar = A, B = 50 μ m, C-H = 20 μ m. Abbreviations: gcl = ganglion cell layer, ipl = inner plexiform layer, inl = inner nuclear layer, opl = outer plexiform layer, onl = outer nuclear layer, is = inner segment, os = outer segment, rpe = retinal pigment epithelium.

Figure 5.

Assessment of ERG components generated by the RPE. dc-ERG responses obtained to a 7-min duration stimulus were obtained from WT (blue) or *cbs*^{+/-} (red) mice at 5 weeks (A) or 30 weeks (B) of age. At 5 weeks, tracings reflect the average of responses obtained from 9 WT or 3 *cbs*^{+/-} mice. All response components were comparable at this age. At 30 weeks, tracings reflect the average of 9 WT or 4 *cbs*^{+/-} responses. At 30 weeks of age, the amplitude of the light peak component was significantly reduced in amplitude ($p < 0.04$). Amplitude scale bar indicates 1 mV.

Figure 6.

VEP changes in *cbs*^{+/-} heterozygous mice. (A) Representative VEP waveforms of 30-week old WT and *cbs*^{+/-} mice obtained to strobe flash stimuli presented to the dark-adapted eye. Arrowheads indicate the implicit time of the main VEP component N1. Vertical dashed lines indicate the time of stimulus flash presentation. Note that the delay observed in response to low intensity stimuli were more pronounced in the *cbs*^{+/-} mouse. Scale bar indicates 20 μ V and 100 ms. (B–D) Intensity-response functions for N1 implicit time obtained at 5 weeks (B), 10 weeks (C) and 30 weeks (D). Data points indicate the average ($\pm$ SD) of 4 WT and 10 *cbs*^{+/-} mice tested at 5 weeks of age, 7 WT and 3 *cbs*^{+/-} mice tested at 10 weeks of age, and 7 WT and 9 *cbs*^{+/-} mice tested at 30 weeks of age. N1 implicit time did not differ between WT and *cbs*^{+/-} mice at 5 or 10 weeks of age. N1 implicit times were significantly delayed in 30 week old *cbs*^{+/-} mice ($p < 0.05$).

Figure 7.

Visual evoked potential in 3 week homozygous mice compared to age-matched heterozygous and wildtype mice. Intensity-response functions for N1 implicit time were obtained from 3-week old WT (blue), *cbs*^{+/-} (red) and *cbs*^{-/-} (black) mice. Note the pattern of VEP delay observed in older *cbs*^{+/-} mice (Fig. 6) is already detectable by 3-weeks in the *cbs*^{-/-} animals.