Progressive retinal degeneration and dysfunction in R6 Huntington’s disease mice

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Huntington’s disease (HD) and spinocerebellar ataxia type 7 (SCA7) belong to a group of progressive neurodegenerative diseases caused by polyglutamine (polyQ) expansions. SCA7 is the only one to display degeneration in the retina, a tissue usually spared in HD. We previously described a SCA7 transgenic retinal model expressing mutant full length ataxin-7 in rod photoreceptors. These mice develop a severe and characteristic retinopathy. We show here that R6 transgenic mice, which reproduce many features of HD, express mutant huntingtin in the retina leading to strong vision deficiencies and retinal dystrophy. These two different polyQ mouse models exhibit comparable early and progressive retinal degeneration and dysfunction. These abnormalities are reminiscent of other retinal degeneration phenotypes (in particular rd7/rd7 mice) where photoreceptor cell loss occurs. Retinopathy in R6 and R7E models can be monitored in living mice by ERG and fundus examination, which can facilitate in vivo evaluation of therapeutic agents in polyQ disorders.

INTRODUCTION

Nine neurodegenerative diseases including Huntington’s disease (HD) and several dominantly inherited spinocerebellar ataxias (SCAs) are caused by an expansion of a polyglutamine (polyQ) stretch in the respective disease-causing proteins. Genetic and molecular studies indicate that expression of polyQ tracts confer a novel toxic function to the otherwise unrelated proteins. Several non-exclusive mechanisms have been proposed to be implicated in polyQ induced toxicity (for a review see refs. 1, 2).

Each of these diseases shows adult-onset neurological symptoms and characteristic post-mortem pathology, with neuronal loss in selected but distinct regions of the central nervous system (CNS). In HD, massive death primarily affects the medium spiny neurons of the striatum, while SCA7 is characterized by neuronal loss within the cerebellum and brainstem. In addition, SCA7 is unique among all polyQ diseases since it shows retinal degeneration. In patients, the retina shows total loss of the outer segments, near-total loss of the inner segments and drastic reduction in the number of nuclei in the outer nuclear layer (ONL) (3,4). The specific neurodegeneration patterns in polyQ diseases are not explained by the widespread expression of the relevant proteins through the brain and non-neuronal tissues. Recent studies provide insights into the molecular basis of cell-specificity, which might be related to dysfunction of cell-specific transcription factors, such as CRX in SCA7 and PQBP-1 in SCA1 (5,6). However, severe juvenile onset cases carrying very large expansions display significant overlap in phenotypes with more widespread brain pathology than observed in typical cases. This indicates that cells that are usually spared can be a target for mutant protein toxic effect.

R6 lines were the first mouse models available for HD and then became the most extensively studied. They were generated by expression of human huntingtin (htt) exon 1 with large (120 to 150) CAG repeat expansions under control of the human HD gene promoter (7). This construct leads to a ubiquitous pattern of expression, including neuronal and non-neuronal tissues (7). Of the different lines obtained, R6/2 presents the most severe neurological phenotype. Behavioral motor deficits can be detected by 5–6 weeks of age but overt phenotypes do not appear until 9 weeks. The progression of the disease is rapid and leads to early death at 10–13 weeks. Movement disorders include resting tremor, seizures, clasping and stereotypic involuntary movements. These mice mimic many features of juvenile HD cases. At death, brain weight is markedly reduced but neuronal density appears normal throughout all CNS...
structures. Staining with antibodies recognizing the transgene product revealed the presence of neuronal intranuclear inclusions (NIs) preceding onset of symptoms (8). Intracellular aggregates of expanded polyQ-containing proteins turned out to be a hallmark of polyQ diseases. R6 mice were used in many different studies aimed at identifying mechanisms of dysfunction and degeneration and strategies to counteract polyQ toxicity.

Two recently developed SCA7 transgenic mouse models have been generated by expressing full length mutant ataxin-7 in the retina (5,9). Both show deficiencies in vision modeling the cone–rod dystrophy observed in SCA7 patients. By using two different promoters, rhodopsin or prion (PrP) formation of NIs are either restricted to the rod photoreceptors or spread throughout the retina. Histological abnormalities in both models are comparable, mainly affecting the photoreceptor nuclear layer and indicate severe progressive degenerative changes in the retina. Of the several other polyQ mouse models generated none were reported to express the transgene in the retina and to develop a retinal phenotype. Because retina is a well characterized CNS tissue that is useful to study inherited neuronal degeneration, we asked whether other expanded polyQ containing proteins could induce a retinopathy or whether only mutant ataxin-7 is toxic in the retina.

We assessed expression of mutant htt in R6 mice retina. Mutant htt exon 1 transgene is expressed in R6/1 and R6/2 retina leading to the formation of NIs throughout the three neuronal layers. Aggregates progressively accumulate and are prevalent when mice exhibit a strong neurological phenotype. Because retina is a well characterized CNS tissue that is useful to study inherited neuronal degeneration, we asked whether other expanded polyQ containing proteins could induce a retinopathy or whether only mutant ataxin-7 is toxic in the retina.

RESULTS

Expression and aggregation of mutant exon 1 htt fragment in the retina of HD transgenic models

We previously generated a mouse model of SCA7 by overexpression of mutant ataxin-7 in the rod photoreceptors (9) (R7E mice). In order to investigate if other polyQ containing proteins could induce a retinopathy, we analyzed retina of the most extensively characterized HD mouse model (7). R6/1 and R6/2 mice were obtained from The Jackson Laboratories (Bar Harbor, Maine), where they are maintained on a mixed C57BL/6.CBA/J genetic background. Since the CBA/J inbred strain carries the rd mutation, causing severe retinal degeneration in the homozygous state, mice were backcrossed on the C57BL/6 background.

Western blot analysis of retinal homogenates using the 1C2 antibody that specifically detects expanded polyQ, showed a high expression of mutant truncated huntingtin at the expected size (80 kDa in R6/2) (Fig. 1A). Expression levels are comparable to that found in brain and were higher in R6/2 retinas than in R6/1 (data not shown). The same blot was reprobed with anti-htt antibody (4C8) whose epitope is not present in the recombinant htt fragment. Endogenous htt was found at similar levels between wild-type and transgenic littermates (data not shown).

Distribution of mutant htt was studied by immunohistochemistry (IHF) on retina cryosections from 10-week-old R6/2 and 32-week-old R6/1 mice. At these ages, animals presented a strong neurological phenotype and several were dying. Confocal analysis revealed mutant htt expression in the three nuclear layers of the retina (Fig. 1B panels a–f). Staining appeared into the main neuronal populations (cone and rod photoreceptors, bipolar and amacrine cells and ganglion cells) as revealed by co-staining with different retinal cell markers (data not shown). As expected, mutant htt overexpression led to early formation of nuclear inclusions (NIs) throughout the three layers of the retina. These aggregates were found to be predominantly nuclear (Fig. 1C). However, mutant htt immunoreactivity was also found in the cytoplasm where it accumulated to form cytoplasmic aggregates in the neuropil. These cytoplasmic aggregates were much less intense and numerous than NIs as shown by the weak staining in the plexiform layers compared to all nuclear layers. NIs size correlated with neurons size with large aggregates found in ganglion cells and smaller one in photoreceptors. Endogenous htt distribution was finally examined by IHF with an antibody distal to exon 1 (4C8). Its expression and localization was not modified in transgenic retinas (data not shown). Endogenous htt was detected as an homogenous cytoplasmic staining highly enriched in both transgenic retinas (data not shown). Endogenous htt was detected as an homogenous cytoplasmic staining highly enriched in both transgenic retinas, which is consistent with its enrichment in synaptic fractions in mouse brain (10). Wild-type htt was not recruited in the NIs.

Fewer aggregates could be observed in the photoreceptor layer (outer nuclear layer: ONL) of R6 mice when compared to R7E mice (Fig. 1B panels g–i). This is likely to be due to a mosaic expression of both transgenes: more than ~90% of photoreceptor cells express the SCA7 transgene product in R7E, whereas we estimated that around 70% of photoreceptor cells express the HD transgene product in R6. In contrast to the restricted rhodopsin promoter driven expression of mutant ataxin-7 in the ONL, NIs were seen in the inner retina (inner nuclear layer: INL and ganglion cell layer: GCL) of R6 mice.

Retinal degeneration and dysfunction

DAPI staining revealed morphological abnormalities in the retina of R6 transgenic animals (Fig. 1B panels b and e). This was analysed more precisely by light microscopy of histological retinal preparations. In contrast to the linear aspect of the different retinal layers in wild-type littermates (Fig. 2A panel a and b), the ONL of R6 mice had a striking irregular and ‘wavy’ shape, disrupted with folds and whorls. Overall ONL thickness does not appear significantly reduced.
Disorganization and reduced thickness of the outer and inner segments were also prominent. Misplaced photoreceptor nuclei were observed in the segment layers and in the OPL (Fig. 2A panels c and d). These anomalies were detected in 10-week-old R6/2 (Fig. 1B panel e) and 32-week-old R6/1 mice (Fig. 1B panel b and Fig. 2A panels c and d). As shown by an anti-htt staining, many NIs were formed at these stages. The peripheral areas of the retina appeared relatively spared despite a similar content in NIs. Overall, this retinal dysplasia covered more than 50% of the entire retina and was presumably due to an extensive degeneration of the photoreceptors.

These histological observations are reminiscent of those seen in R7E mice. In particular, no obvious abnormalities were detected in the inner plexiform layer (IPL) and GCL of both models. The INL anomalies looked similar and were likely to be secondary to ONL irregular shape. Despite these R6 mice appeared to show a preserved inner retina, neurons in the GCL and the INL contained large and numerous aggregates (Fig. 1C).

To evaluate retinal function in vivo, we recorded electroretinograms (ERGs) with various light stimulus intensities under dark-adapted scotopic and light-adapted photopic conditions, from transgenic and wild-type littermate mice. Scotopic ERGs record both rod photoreceptors (a-wave) and rod-initiated post-synaptic responses (b-wave) and photopic ERGs record both cone photoreceptors (a-wave) and cone-initiated responses (b-wave). ERG recordings from 32-week-old R6/1 mice showed an overall dysfunction of the retina, with
reduction of both a- and b-waves at maximal intensities (see Materials and Methods) (Fig. 2A panels e and f).

Scotopic and photopic ERG intensity series recordings showed a dysfunction of rod and cone pathways, respectively (Fig. 2B). The a-wave amplitude at the maximum intensity was reduced by 50% in transgenic mice (96 μV/C69; n = 8) with respect to control wild-type littermate mice (195 μV/C622; n = 7) (Fig. 2B panel a). This reduction in the ERG a-wave amplitude indicated that rod function was greatly affected. At the maximum intensity, the ERG b-wave was reduced by 70% in transgenic mice (118 μV/C613; n = 8) with respect to control wild-type littermate mice (350 μV/C656; n = 7) (Fig. 2B panel b). When the b-wave/a-wave ratio was calculated, it fell to 1.29 (±0.2; n = 8) in transgenic mice whereas this ratio was measured at 1.76 (±0.22; n = 7) in control mice. Such a great decrease in the b-wave amplitude compared with that of the a-wave indicated that rod bipolar cells postsynaptic to photoreceptors were also strongly functionally altered. Finally, the cone pathway was tested by measuring the photopic responses. In R6 animals, ERG responses were completely suppressed (Fig. 2B panel c) indicating that cones and their post-synaptic neurons were also severely affected by the transgene expression.

We then used a simpler non-invasive procedure to evaluate the phenotype on living animals. Clinical examination of R6/1 at 32 weeks and R7E mice at 10 weeks of age by indirect ophthalmoscopy revealed a similar phenotype with the presence of evenly spaced white spots across the retina (Fig. 2A panels g–j).

Histology, ERG recordings and fundus examination from Figure 2A were performed on the same animal, either wild-type (in Fig. 2A panels b, e and g) or transgenic (in Fig. 2A panels d, f and h). This highlights a clear correlation between functional abnormalities and neuropathological features in R6 mice.

**Figure 2.** Retinal degeneration and dysfunction in R6 mice. (A) Light microscopy, ERG and fundus photography of 32-week-old transgenic R6/1 (lower panels) and wild-type control littermate (upper panels) mice. Histology reveals outer nuclear layer wavy aspect and shrinkage of the outer segments in R6/1 when compared to wild-type retinas (compare panels c and d to a and b). Thin (6 μm) sections were stained with hematoxylin/eosin. ERG response at high intensities (from 0 to 1.4 log cd s m⁻²) is largely reduced in R6/1 when compared to control mice, showing dysfunction of photoreceptors and of the downstream neurons (compare panel f to e). Finally, both R6/1 (panel h) and R7E (panel j) mice have white spots over the retina (arrows) which were absent in control littermate mice (panels g and i, respectively). Panels b, c, g and d, f, h correspond to histology, ERG and fundus of the same wild-type and transgenic animal, respectively. The retinal detachment in a–d is artefactual and due to tissue processing. RPE, retinal pigmentary epithelium; OS, outer segments; IS, inner segments; ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer. (B) Intensity-response curves of the dark-adapted (a, b) and light-adapted (c) ERG a- (a, c) and b- (b, c) waves for seven wild-type and eight R6/1 transgenic animals at 32 weeks of age. Individual symbols represent means±SEM of the different groups on each recordings and stimuli. ANOVA (see Materials and Methods) demonstrates significant effect of the genotype on rod electrophysiological response (panel a, F₁,13 = 17.68, P = 0.001), on rod-initiated response (panel b, F₁,13 = 15.22, P = 0.002), on cone response (panel c, F₁,13 = 19.23, P = 0.001) and on cone-initiated response (panel c, F₁,13 = 43.35, P < 0.001).
Furthermore, fundus examination provides an useful means to assess the pathology in living R6 animals and also in R7E mice.

**Retinopathy progression**

In order to evaluate the progression of this retinal phenotype, we analysed R6/1 animals at 10, 16 and 32 weeks of age by histology, ERGs and/or fundus examination. These ages pre-cede or correspond to the onset of neurological symptoms and first deaths observed in this line, respectively (7). As expected the retinopathy had a late onset and was progressive. Indeed, light microscopy of retinas from young (10-week-old) R6/1 transgenic animals showed no abnormalities. The ONL displayed a perfect linear aspect with no obvious sign of degeneration (Fig. 3A). First histological abnormalities were detected at around 16 weeks of age (data not shown).

ERG recordings of transgenic animals at this age already revealed moderate abnormalities. The scotopic ERG demonstrated a functional rod pathway (Fig. 3B panels a and b), while the photopic ERG suggested an impairment of the cone-initiated response (Fig. 3B panel c). However, the difference observed at this age was not statistically significant as one animal had a normal light-adapted ERG b-wave and the other had a flat response. This probably reflects a variability between mice in the onset of neurological symptoms. The same mice were then reanalysed 4 months later. As expected, transgenic R6/1 showed a strong reduction of dark-adapted ERGs (Fig. 3B panels a and b) and a flat response for light-adapted ERGs (Fig. 3B panel d). Similarly, fundus examination of R6/1 was normal at 4 months of age whereas the same animal displayed numerous white retinal spots at 8 months (Fig. 3C).

**Immunoreactivity of htt aggregates**

Several studies suggest that polyglutamine aggregates recruit different proteins such as chaperones or transcription factors and might even deplete them from their normal localization. To investigate their potential role in polyQ retinal degeneration, we looked at different candidate proteins focusing on those highly expressed in the retina. Particularly, in normal conditions, photoreceptors show high levels of inducible Hsp70 which is barely detectable in other tissues. We thus examined NIs immunoreactivity of R6 retinal sections and steady-state protein levels by western blotting of retinal homogenates (results are summarized in Table 1). Ubiquitinated NIs recruited Hdj1, Hdj2 and Hsc70 without altering either their normal localization or the expression levels of their soluble form. Crx immunoreactivity was not found in R6 NIs but appeared as an homogenous nuclear staining similar to that found in wild-type retinas.

Polyglutamine protein interaction with CBP has been extensively studied and suggests that depletion of soluble CBP could result in altered gene expression (11,12). We found equal levels of soluble CBP in R6 and control retinas (Fig. 4A) despite a strong co-localization in aggregations from all neuronal populations (Fig. 4B panels a–c). Strikingly, out of the different proteins analysed Hsp70 was the only one quantitatively altered. Western blotting of R6 retinal homogenates showed around 40% reduction of Hsp70 level (Fig. 4A). In contrast, it was never found recruited in R6 NIs (Fig. 4B panels d–f) even in the photoreceptors which displayed a high Hsp70 immunoreactivity in both wild-type and transgenic mice.

Our results do not support a model of pathogenesis in which recruitment of transcription factors and chaperones in polyQ NIs reduce their relative levels. CBP and chaperones like Hsp40 and Hsc70 localized in htt aggregates without being depleted, while Hsp70 was downregulated but not recruited in NIs.

**DISCUSSION**

We observed a strong retinal phenotype in R6/1 and R6/2 mice. Overexpression of mutant htt exon 1 led to early formation of NIs in the three neuronal layers of the retina. Electrophysiological abnormalities in the retina were detected at the time of appearance of the neurological symptoms. ERG recordings indicated that cones are affected earlier than rods and that cone- and rod-induced responses were severely reduced. Neurodegeneration in the retina was suggested by the abnormal morphology of the photoreceptor layer and numerous low cellular density areas in the ONL. Retinal dysplasia was detected as white spots across the retina by fundus examination of living mice. This phenotype was progressive, eventually leading to major visual impairment of the R6 mice. Finally we observed a two-fold downregulation of Hsp70 level that could be implicated in the polyQ induced retinal phenotype.

**Comparison of R6 and R7E retinopathies**

We observed a similar retinal phenotype in HD and SCA7 mouse models which express a fragment of huntingtin (htt) in the whole retina (R6) or full length ataxin-7 exclusively in the rods (R7E), respectively. Molecular and phenotypic events appeared at around 1 to 2 months of age, and progressively worsened in both R6/2 and R7E retina. In both models ONL wavy shape was the most striking feature, while the inner retina seemed spared. In contrast ERG recordings were different: R7E...
retinal dysfunction was mainly restricted to the photoreceptors with a specific reduction of the a-wave while in R6 animals both a- and b-waves were affected. This suggests a dysfunction of the inner retina which shows no obvious histological sign of degeneration in the HD model.

Cell-specificity of polyQ-induced toxicity

Among all polyQ disorders, SCA7 is the only one in which primary sites of neurodegeneration include the retina. Our study shows that retinopathy can be induced in mouse by polyQ expansion in two unrelated proteins. Two SCA7 mouse models have been generated using either the rhodopsin promoter or the PrP promoter and exhibit a comparable retinal phenotype. Surprisingly, a HD mouse model generated using its own promoter leads to similar retinopathy. The ability of mutant htt to induce cell dysfunction in retinal neurons not reported to degenerate in HD is intriguing. However over-expression of a N-ter fragment of htt with 150 Q is highly toxic and affects various cell types including non-neuronal tissues. NIs were observed in muscle and pancreatic cells of R6 mice, which were reported to develop diabetes (13,14). R6 mice also develop many of the features of juvenile HD known to show more widespread pathology than adult onset cases. Furthermore, signs of retinal impairment have been reported in few HD/SCA patients extending the clinical definition of these polyQ diseases. Impairment of retinal increment thresholds in HD patients suggest that the retina might be affected (15).

Isolated cases of MJD/SCA3 and SCA2 present with retinal degeneration (16,17), which however could be an incidental finding in the SCA3 patient. The expansion of more than 200 repeats found in the SCA2 patient suggests that larger expansions widen the pathology.

The molecular basis of cell-specific degeneration pattern in polyQ diseases is not clearly understood. Insight into the retinal specific degeneration has been provided by La Spada et al. suggesting that mutant ataxin-7 disrupts the normal function of the photoreceptor specific transcription factor Crx. It would then be interesting to assess whether R6 retinopathy is also mediated by a disruption of Crx normal activity. In that case, Crx function would be impaired by other unrelated polyQ proteins and could not account on its own for the SCA7-restricted retinopathy. In the other case, polyQ toxic effect in the retina could appear without alteration of Crx normal function. Many different pathways are likely responsible for polyQ induced toxicity (1) and a subtle balance...
leads to an excess cone cell proliferation causing this specific retinal dysplasia (22). N2e3 is involved in photoreceptor differentiation working together with other transcription factors such as Crx and Nrl (23–25). Homozygous deletion of Nrl in mice results in a comparable disruption of the ONL and shrinkage of the segments (26). Together these findings suggest that the Nrl pathway and photoreceptor cell fate might be altered in R7E and R6 mice retina.

**Modeling polyQ toxicity in the mouse retina**

Our study shows that neuropathological features in R6 mice affect the retina. Up to now the R6 neurological phenotype was not reported to be associated with overt neuronal pathology. Neuronal death is minimal and delayed compared to the behavioral symptoms (8,27). Histological abnormalities of R6 retina and retinal dysfunction assessed by ERG provide evidence of neurodegeneration and demonstrate a visual impairment of R6 mice. Although we did not precisely characterize the onset of this phenotype, behavioral tests based on visual acuity of R6 mice should be then carefully interpreted.

Retina is useful for molecular analysis of neurodegeneration as it is a highly organized and homogenous tissue with a limited number of different cell types. In particular polyQ induced downregulation of Hsp70 could be evidenced in this tissue and would have been more difficult to assess in other CNS regions. Moreover, retina is a suitable tissue for gene transfer and drug delivery, offering new opportunities to test therapeutic strategies in polyQ diseases. Both R7E and R6 mice display an early onset and rapid progression of the retinopathy. ERG and funduscopic examinations both provide a non invasive and reproducible phenotypic evaluation in living animals.

**MATERIALS AND METHODS**

**Animals**

R7E transgenic line was maintained on the inbred C57BL/6 background (9). R6/1 and R6/2 lines (7) were obtained from The Jackson Laboratories (Bar Harbor, Maine). In this study, mice were backcrossed on the inbred C57BL/6 background. For genotyping of R7E and R6 mice, mouse tail DNA was screened by PCR according to the procedures previously described in (9) and in (7), respectively. The loss of the rd allele from the SJL (R7E founders) or from the CBA backgrounds was checked by PCR and Dpnl digestion (29).

**Western blotting**

Retinas were dissected and homogenized in lysis buffer containing 50 mM Tris–HCl pH 8.0, 10% glycerol, 5 mM EDTA, 150 mM KCl, 1 mM phenylmethylsulfonyl fluoride, a cocktail of protease inhibitors. Nonidet® P40 was then added, to a final concentration of 1%, to whole retinal homogenates, which were then incubated for 20 min on ice and centrifuged for 30 min at 10 000 g at 4°C. Supernatants were analysed on an 8 or 10% SDS–PAGE gel. Primary antibodies used were:
mouse monoclonal anti-htt 1C2 at 1:2000; mouse monoclonal anti-htt 4C8 at 1:1000; mouse monoclonal anti-β-actin (Abcam, Cambridge, UK); mouse monoclonal anti-dynamin Hudy-2 (Upstate Biotechnology, Lake Placid, NY); mouse monoclonal anti-Hdj-1/Hsp40 (MBL, Japan); mouse monoclonal anti-Hdj-2/Hsp40 (Neomarkers, Fremont, CA); mouse monoclonal anti-Hsc70 SPA-815 (Stressgen, Victoria, Canada); mouse monoclonal anti-Hsp70 SPA-810 (Stressgen, Victoria, Canada); rabbit polyclonal anti-CBP A-22 (SCB, Santa Cruz, CA); mouse monoclonal anti-TPB 3G3 at 1:1000 (gift from L. Tora); mouse monoclonal anti-ataxin-3 1H9 at 1:1000. All were revealed with appropriate anti-mouse or anti-rabbit peroxidase-conjugated secondary antibodies (Jackson ImmunoResearch Laboratories, West Grove, PA) and the ECL chemiluminescent reaction (Pierce, Rockford, IL).

Immunohistology and light microscopy

For histology, enucleated eyes were fixed by immersion in Bouin for 12 h at room temperature or in 4% paraformaldehyde, 0.3 mM CaCl2, 0.1 mM MgCl2, 1x PBS, for 12 h at 4°C. Fixed eyes were rinsed in 1x PBS twice, dehydrated with a graded alcohol series and embedded in paraffin. Semi-thin section (6 μm) were stained with hematoxylin and eosin or with carbolated toluidin blue, as stated.

For immunohistofluorescence, enucleated eyes were dissected to remove lens and cornea and fixed in fresh 4% paraformaldehyde, 0.3 mM CaCl2, 0.1 mM MgCl2, 1x PBS, for 2 h at 4°C. Fixed retinas were placed for 1 h in 30% sucrose, 1x PBS and frozen in OCT compound. Cryostat sections (10 μm) were mounted on SuperFrost/Plus slides (O. Kindler, Freiburg, Germany), permeabilized for 5 min with 0.1% Triton X-100, 1x PBS, blocked for 15 min with 0.5% bovine serum albumin, 0.1% Tween-20, 1x PBS. Primary antibodies used were: mouse monoclonal anti-htt 1259 at 1:100 (30); rabbit polyclonal affinity-purified anti-axin-7 1261 at 1:100 (9); mouse monoclonal anti-Hsp70 SPA-810 (Stressgen, Victoria, Canada); rabbit polyclonal anti-ubiquitin (Sigma-Aldrich, Saint Louis, MO); mouse monoclonal anti-Hdj-1/Hsp40 (MBL, Japan); mouse monoclonal anti-Hdj-2/Hsp40 (Neomarkers, Fremont, CA); mouse monoclonal anti-Hsc70 SPA-815 (Stressgen, Victoria, Canada); mouse monoclonal anti-Hsp70 SPA-810 (Stressgen, Victoria, Canada); rabbit polyclonal anti-CBP A-22 (SCB, Santa Cruz, CA); mouse monoclonal anti-TPB 3G3 at 1:100 (gift from L. Tora); mouse monoclonal anti-ataxin-3 1H9 at 1:1000. All were revealed with appropriate anti-mouse or anti-rabbit peroxidase-conjugated secondary antibodies (Jackson ImmunoResearch Laboratories, West Grove, PA) and the ECL chemiluminescent reaction (Pierce, Rockford, IL).

Electroretinography

Mice were dark-adapted for 24 h and anesthetized with intraperitoneal injection (25 μl/g body weight) of Hypnovel (5 mg/ml) and Etomidate (2 mg/ml). Mice were clinically evaluated by indirect ophthalmoscopy with a Kowa Genesis small animal fundus camera (Tokyo, Japan) and a condensing Volk 90-diopter lens (Mentor,OH, USA) mounted between camera and eye. Fundi were examined through pupils dilated with 0.05% Tropicamide or 1% Atropine. Fundus photographs were taken with Kodak Elite 200 ASA print film.

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