岩手医科大学
審査学位論文
（博　士）

Contribution of N-Methyl-DL-Aspartic Acid (NMDA)-sensitive Neurons to Generating Oscillatory Potentials in Royal College of Surgeons Rats

Authors: Tomomi Harada, Shigeki Machida, Tomoharu Nishimura, Daijiro Kurosaka

Affiliation: Departments of Ophthalmology,
Iwate Medical University School of Medicine
19-1 Uchimaru, Morioka, Iwate 020-8505, Japan

Corresponding author: Shigeki Machida MD, PhD
Department of Ophthalmology, Iwate Medical University
School of Medicine
19-1 Uchimaru, Morioka, Iwate 020-8505, Japan
TEL: 81-19-651-5111 (Ex. 8691)
FAX: 81-19-653-2864
E-mail: smachida@iwate-med.ac.jp

Running title: OPs of RCS rats

Words: 3,172

Figures: 7

Tables: 0

Category: Original Research Article

Grant support: Supported by a Grant (#17591850) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (SM).

ABSTRACT

Purpose: We investigated how the N-Methyl-DL-Aspartic Acid (NMDA) receptor contributes to generating oscillatory potentials (OPs) of the electroretinogram (ERG) in the Royal College of Surgeons (RCS) rat.

Methods: Scotopic ERGs were recorded from dystrophic and wild-type congenic (WT) RCS rats (n=20 of each) at 25, 30, 35, and 40 days of age. The stimulus intensity was increased from -2.82 to 0.71 log cd-s/m^2 to obtain intensity-response function. NMDA was injected into the vitreous cavity of the right eyes. The left eyes were injected with saline as controls. The P3 obtained by a-wave fitting was digitally subtracted from the scotopic ERG to isolate the P2. For the oscillatory potentials (OPs), the P2 was digitally filtered between 65 and 500 Hz. The amplitudes of OP1, OP2, OP3, and OP4 were then measured and summed and designated as ΣOPs. The implicit times of OP1, OP2 and OP3 were also measured. The frequency spectra of the OPs were analyzed using fast Fourier transform (FFT).

Results: The ERG a- and b-waves as well as ΣOPs amplitudes reduced with age in dystrophic rats. Compared with intravitreal saline injection, administration of NMDA decreased ΣOPs amplitudes from 30 days of age in dystrophic rats, while it did not attenuate ΣOPs amplitudes in WT rats. The implicit times of the OPs were prolonged by NMDA injections in WT and dystrophic rats. NMDA/saline ratio of area under the FFT curves was significantly lower in dystrophic rats from 30 days of age than that in WT rats.

Conclusion: In the early stage of photoreceptor degeneration, intravitreal NMDA injection attenuated OPs amplitudes in dystrophic rats. This indicates that NMDA

receptors play a significant role in generating OPs amplitudes with advancing photoreceptor degeneration.

Key words: RCS rat, ERG, OPs, oscillatory potentials, photoreceptor degeneration

INTRODUCTION

The Royal College of Surgeons (RCS) rat has been widely used in the research field as a model of the human disease, retinitis pigmentosa (RP) [1]. A recessive mutation of the receptor tyrosine kinase gene (*Mertk*) has been shown to prevent retinal pigment epithelium (RPE) cells from phagocytosing shed photoreceptor outer segments [2]. The *Mertk* knockout mouse has been successfully bred to have the same phenotype as the RCS rat [3]. In addition, transfer of the *Mertk* gene to the diseased RPE cells of the dystrophic RCS rat restored phagocytosis of the RPE *in vitro* and *in vivo* [4, 5]. Recently, this mutation has been identified in patients with autosomal recessive RP [6, 7]. Thus, the dystrophic RCS rat is gaining importance in research aimed at understanding the pathophysiology of or developing new treatments for RP [8, 9].

The electroretinogram (ERG) is a very useful and non-invasive tool to measure retinal function in animals and humans with retinal disease. The third-order neuronal responses including the scotopic threshold response (STR) [10] and photopic negative response (PhNR) [11] were preserved or enhanced in dystrophic RCS rats, despite progressive loss of photoreceptors [12-14]. This indicates that functional alternation of the third-order neuronal response takes place with photoreceptor degeneration in these rats.

Oscillatory potentials (OPs) consist of a series of small wavelets appearing on the ascending limb of the ERG b-wave. Although the origin of OPs remains undetermined, the inner retina likely generates this response [15]. OPs have been widely used to evaluate inner retinal function in clinical and animal research [15-17]. In photoreceptor diseases in which the inner retina is assumed to be intact in the early stages, OPs show unexpected changes. In transgenic swine and rabbit models of photoreceptor degeneration with the rhodopsin

P347L mutation, OPs of the cone ERG are enhanced in the early stage of degeneration [18, 19]. In some patients with RP whose visual function was relatively preserved at the posterior pole of the retina, OPs of the focal macular ERG were reported to be supernormal [20]. These suggest that the OPs could be abnormal in the early stage of photoreceptor degeneration.

We have shown that the ERG waveforms of RCS rats change dramatically after intravitreal injection of N-methyl-DL-aspartic acid (NMDA) [13, 14]. The b-wave amplitude increases and the threshold decreases with elimination of the STR and PhNR in RCS rats, while these findings are not apparent in normal rats. This suggests that a NMDA-sensitive component, the third-order neuronal response [21], dominates the ERG in dystrophic RCS rats.

Since the dystrophic RCS rat is an important animal model of inherited photoreceptor degeneration [8, 9], it is vital in the understanding of ERG characteristics as this degeneration advances. In the present study, we investigated how OPs change with photoreceptor degeneration and how intravitreal injection of NMDA affects the OPs in dystrophic and wild-type (WT) congenic RCS rats.

METHODS

Animals

Dystrophic and WT congenic RCS rats were obtained from CLEA Japan (Tokyo, Japan) at 21 days of age. They were all albinos. Dystrophic rats were homozygous for the retinal dystrophy gene. A total of 25 dystrophic and 25 WT rats were housed in our

laboratory with a 12:12 hour light-dark cycle and illumination at 5 lux until the experiments.

All animals were housed and handled with the authorization and supervision of the Institutional Animal Care and Use Committee of the Iwate Medical University. All procedures conformed to the Association for Research in Vision and Ophthalmology Resolution on the Use of Animals in Ophthalmic and Vision Research.

Recording of ERGs

Scotopic ERGs were recorded from both eyes of dystrophic and WT RCS rats at 25, 30, 35, and 40 days of age. Five dystrophic and five WT rats were used at each time point, and all animals were sacrificed after the first ERG recording. Therefore, we recorded ERGs from different animals at each time point. A total number of animals used throughout the experiment was 20 for either dystrophic or WT rats. For the ERG recordings, the animals were dark-adapted overnight and prepared under dim red light. They were anesthetized by a single intramuscular injection of a mixture of ketamine (87 mg/kg) and xylazine (13 mg/kg). The pupils were maximally dilated by topical 0.5% tropicamide and 0.5% phenylephrine HCl, and corneal anesthesia was achieved with topical 0.4% oxybuprocaine hydrochloride.

Gold contact lens electrodes were placed on the cornea as active electrodes, and needle electrodes were placed subcutaneously at the forehead as reference electrodes. An aluminum sheet under the animals served as the ground electrode. Responses were amplified 10^5 times and bandpass-filtered from 0.3 to 500 Hz (PC-100, Mayo, Nagoya, Japan). The intensity of the stimuli was increased from -2.82 to 0.71 log cd-s/m^2 in 0.53 or 0.47 log unit steps. The duration of stimuli was 10 μs. Two or three

responses were digitally averaged when necessary with an inter-stimulus interval ranging from 10 seconds to 2 minutes depending on the stimulus intensity.

Extraction of OPs (Figure 1)

To reduce contamination of the early portion of the OPs by the a-wave, we applied the method reported by Akula et al. [22] for isolating the OPs. First, the leading edge of the scotopic a-waves was fitted by the following equation developed by Hood and Birch [23] , which had been originally applied to a single photoreceptor [24] (Figure 1A).

$$P3(i, t)=\{1-\exp[-i \cdot S(t - t_d)^2]\}Rm$$

where i is the stimulus intensity; td is the time delay; t is the time after stimulus onset; S is sensitivity; and Rm is maximum response amplitude. Second, we subtracted P3 from the original waveform to obtain P2 which mainly consists of the post-receptoral response.

Since the lower frequency end of OPs appears at 65 Hz in rats, as previously reported, the P2 was digitally filtered between 65 and 500 Hz [25]. Finally, the amplitudes of OP1, OP2, OP3, and OP4 were measured, summed, and designated as ΣOPs as previously reported (Figure 1B) [26]. One may concern contribution of positive OFF-responses to the OPs. Since the positive OFF-response is very small and negligible in rodents [27, 28], it does not likely shape main components of the OPs in rats.

The frequency spectra of the OPs were analyzed using fast Fourier transform (FFT) with a filter which bandpassed 65-500 Hz. The lower and upper limit of the bandpass filter was 32.5 (65/2) and 750 (500×1.5) Hz, respectively.

Drug administration

To investigate how NMDA receptors contribute to generating the OPs, NMDA was injected into the vitreous cavity of dystrophic and WT rats. NMDA suppresses synaptic transmission between the bipolar cells and third-order neurons [21]. NMDA was dissolved in saline with the pH adjusted to 6.8-7.2 with 1N NaOH. Intravitreal injections of 2 μl were administered through a 30-gauge needle inserted approximately 1 mm behind the corneal limbus using a Hamilton 80001 syringe (Hamilton, Reno, NV). The approximate intravitreal drug concentration, 5 mM NMDA, was estimated by assuming full mixing in 38 μl vitreous volume [27]. As controls, the left eyes were injected with saline in the same manner. ERG was conducted between 30 and 90 minutes after the intravitreal injections.

Statistical analyses

Two-way factorial ANOVA was applied to determine the statistical significance of differences between NMDA- and saline-injected eyes in changes of the amplitudes, implicit times and sensitivity with age. Two-way repeated measure ANOVA was used to compare the intensity-response curves of the NMDA-injected eyes to those of the saline-injected eyes. In addition, Bonferroni post hoc test was performed following the ANOVA to determine statistical significance between paired data at each time point. The area under the curve (AUC) was used to compare the FFT curves. These analyses were performed using the software SPCANA 4.91 (Fukuyama University, Fukuyama, Japan) and Prism 5.1 (GraphPad Software Inc. San Diego CA). Statistical significance was set at $P < 0.05$.

RESULTS

Age-related ERG changes in dystrophic and WT congenic RCS rats

Age-related ERG changes in saline-injected control eyes of WT (black filled circles) and dystrophic rats (black filled squares) are shown in Figure 2. In these figures, averaged ERG amplitudes and ΣOPs/b-wave amplitude ratio were plotted against days of age. Although the ERG amplitudes remained unchanged in WT rats throughout the experimental period, they declined with advancing age in dystrophic rats (P<0.0001, Figure 2A-C). There was no significant changes with age in the ΣOPs/b-wave amplitude ratio for WT and dystrophic rats (Figure 2D).

Waveform changes after intravitreal injections of NMDA

Representative ERG waveforms of saline-injected (black lines) and NMDA-injected eyes (red lines) are shown for WT and dystrophic rats at 25 and 30 days of age in Figure 3. Although there was no considerable change in the a-wave amplitude and implicit time following NMDA injections in WT and dystrophic rats, the b-wave amplitude was slightly increased in NMDA-injected eyes compared to that in saline-injected eyes in dystrophic rats.

A close look at the extracted OPs reveals that the implicit time was prolonged in NMDA-injected eyes compared with saline-injected eyes of WT and dystrophic rats. In addition, at day 30, OP amplitudes were considerably reduced in the dystrophic rat in the NMDA-injected eye. In WT rats, the OP1amplitude was enhanced whereas the OP2 and OP3 amplitudes were reduced by NMDA application, although the sum of OPs amplitudes appeared unchanged.

Comparison of ERG amplitude between NMDA- and saline-injected eyes

Red symbols in Figure 2 represent averaged ERG amplitudes and ΣOPs/b-wave amplitude ratio of NMDA-injected eyes throughout the study period for WT (red open circles) and dystrophic rats (red open squares). No significant difference was found in a-wave amplitude between NMDA- and saline-injected eyes in WT and dystrophic rats at any age (Figure 2A).

In WT rats, NMDA injections did not significantly affect ERG amplitude or ΣOPs/b-wave amplitude ratio. On the other hand, in dystrophic rats, changes of the b-wave amplitude with age was significantly different between NMDA- and saline-injected eyes $P<0.005$) with larger amplitudes in NMDA-injected eyes from 30 days of age (Figure 2B), consistent with our previous results [13].

Changes of the ΣOPs amplitude with age was significantly different between NMDA- and saline-injected eyes (Figure 2C, $P=0.0001$) with a significant reduction in NMDA-injected eyes at 35 ($P<0.001$) and 40 days of age ($P<0.05$) in dystrophic rats. The ΣOPs/b-wave amplitude ratio differently changed with age between NMDA- and saline-injected eye (Figure 2D, $P<0.0001$) with significant lower values at 30 ($P<0.001$) and 35 days of age in the NMDA-injected eyes ($P<0.01$) in dystrophic rats.

Although no difference was found in the ΣOPs/b-wave amplitude ratio of saline-injected eyes between WT and dystrophic rats as mentioned earlier, in NMDA-injected eyes the ratio was significantly lower in dystrophic rats than that of WT rats from 30 days of age (Figure 2D, $P<0.001$). This indicates that preservation of the OP amplitude disappeared after NMDA injections in dystrophic rats.

Comparison of intensity-response curves of ΣOPs amplitudes between NMDA- and saline-injected eyes

ΣOPs amplitudes were plotted against stimulus intensities to obtain intensity-response curves for NMDA- and saline-injected eyes of WT and dystrophic rats. Figure 4A shows averaged values of intensity-response curves of 35-day-old rats. In WT rats, there were no differences in the intensity-response function between the NMDA- and saline-injected eyes. On the other hand, in dystrophic rats the intensity-response curve of the NMDA-injected eyes was significantly different from that of the saline-injected eyes with lower amplitudes in NMDA-injected eyes except for 25 days of age (P<0.05).

The sensitivity of the ΣOPs was taken to be the stimulus intensity necessary to elicit one-half of the maximum amplitude of the ΣOPs. From the intensity-response curve of each animal, the sensitivity of the ΣOPs was obtained for each animal and then averaged for NMDA and saline-injected eyes of WT and dystrophic rats at all ages (Figure 4B). The sensitivity of the dystrophic rats significantly decreased with age (P<0.0001) without difference between the NMDA- and saline-injected eyes. The intensity-response curves of the WT rats shifted leftward in the low intensity range by NMDA injections (Figure 4A), resulting in significantly higher sensitivities in NMDA-injected eyes compare to the saline-injected eyes (Figure 4B, P<0.05 at 25 and 30 days of age).

Comparison of OP implicit time between NMDA- and saline-injected eyes

To quantitatively evaluate changes of the OPs implicit time, we measured the implicit time of each OP wavelet for the NMDA- and saline-injected eyes (Figure 5A). The implicit time of all OP wavelets significantly prolonged with age in dystrophic rats (Figure 5B-D, P<0.0001), while it unchanged in WT rats. NMDA injections delayed the implicit time of all OP wavelets in WT and dystrophic rats. Changes of the implicit times with age was significantly different between NMDA- and saline injected eyes of WT (P<0.0001) and

dystrophic rats (Figure 5B-D, $P<0.05$ for the OP1, $P<0.005$ for the OP2, $P<0.0001$ for the OP3).

AUC of FFT curves

Figure 6A represents FFT curves obtained from NMDA-(red lines) and saline-injected (black lines) eyes of WT and dystrophic rats at 30 days of age. In the WT rat, the curve of the NMDA-injected eye shifted toward lower frequency (i.e., to the left) compared with that of the saline-injected eye, although peak amplitude and curve configuration remained unchanged. In the dystrophic rat, NMDA application also shifted the FFT curve toward the left. Unlike in the WT rat, peak amplitude was reduced in the NMDA-injected eyes, and this was accompanied by depressed curve configuration. These findings were not only observed on day 30 but remained on days 35 and 40 of age.

Averaged values of peak frequency are shown in Figure 4B for NMDA- (red symbols) and saline-injected eyes (black symbols) of WT (circles) and dystrophic RCS rats (squares). Changes of the peak frequency with age was significantly different between NMDA- and saline-injected eyes in WT and dystrophic rats ($P<0.05$). Figure 6C represents averaged AUCs of FFT curves for NMDA- and saline-injected eyes of WT and dystrophic rats. We found no significant difference in AUC between NMDA- and saline-injected eyes for WT and dystrophic rats.

NMDA / saline ratio of ΣOPs amplitudes and AUCs of FFT curves

The ratios of variables for NMDA- and saline-injected eyes (NMDA / saline) were calculated for ΣOP amplitudes and AUCs of FFT curves, and compared between WT (black bars) and dystrophic rats (white bars) at different ages (Figure 7). These ratios were around

1.0 at all ages in WT rats, indicating that intravitreal injection of NMDA did not alter OP amplitude or AUC of FFT curves. On the other hand, in dystrophic rats, these ratios were significantly lower in the dystrophic rats than in the WT rats, with the exception of day 25 ($P<0.05$).

DISCUSSION

In the present study, we showed that OP amplitudes were reduced by intravitreal injection of NMDA in dystrophic rats, while they were not attenuated in age-matched WT rats. This phenomenon was pronounced after 30 days of age in dystrophic rats, in which photoreceptor degeneration had already taken place. This indicates that photoreceptor degeneration could modify the function of NMDA receptors in generating OPs in the inner retina.

Role of NMDA receptors in generating OPs

NMDA receptors are reported to be mainly expressed in the inner retina [21]. Vaegan and Millar [30] demonstrated that intravitreal injections of NMDA moderately reduced amplitudes of the OPs without affecting their timing in cats. In contrast, our results show that summed amplitudes of the OPs were not attenuated by intravitreal NMDA, while their implicit times appeared to be prolonged in WT rats, indicating differences in pharmacological effects of NMDA on the OPs between these species.

In the normal rat retina, NMDA modifies the timing and frequency of the OPs without changing the sum amplitude. However, in the diseased retina with photoreceptor degeneration, the NMDA receptor also plays a role in determining OP

amplitude. At 25 days of age, when most photoreceptors are still preserved [1] even in dystrophic rats, the OP sum amplitude was not affected by intravitreal injections of NMDA. This indicates that the role of NMDA receptors in the inner retina changes with advancing photoreceptor degeneration.

OP changes in other photoreceptor degeneration models and in the clinical setting

The preservation of the OP amplitude disappeared after intravitreal injections of NMDA in dystrophic rats, indicating that it stems from NMDA-sensitive neurons. In other animal models of inherited photoreceptor degeneration, such as P347L rhodopsin transgenic swine and rabbits, supernormal OPs were observed in the early stage of photoreceptor degeneration [18,19]. In P347L rhodopsin transgenic rabbits, an intravitreal injection of tetrodotoxin (TTX), which blocks the voltage-gated sodium channels of the retinal ganglion cells (RGCs) and amacrine cells [31-33] eliminated enhancement of the OPs. This indicates that TTX-sensitive neurons contributed to the supernormal OPs in the rabbit model. In the clinical setting, OPs of the focal macular ERG were preserved or enhanced in some RP patients with good vision [19]. All this evidence suggests that the inner retinal function reflected by the OPs is preserved or enhanced in the early stage of photoreceptor degeneration.

An NMDA-sensitive component dominates ERG in RCS rats

Our results show that NMDA-sensitive neurons have a greater role in generating OP amplitudes in dystrophic rats than in WT rats. Our previous results demonstrated that STR and PhNR, which are also NMDA-sensitive components, dominate the scotopic and photopic ERGs in dystrophic RCS rats [12-14]. Taken together, this evidence

indicates that an NMDA-sensitive component of the ERG has a greater contribution to shaping the waveform in dystrophic rats than in their WT counterparts. However, this finding is not specific to dystrophic RCS rats. We also found that an NMDA-sensitive component demonstrated a considerable contribution in shaping the ERG in sodium iodate-induced retinopathy soon after induction of the degeneration [34].

It remains unclear why an NMDA-sensitive component dominates the OPs of the ERG in RCS dystrophic rats. However, the following explanations are possible. First, retinal remodeling occurring in the middle and inner retinal layers is triggered by photoreceptor loss, which is independent of the initial molecular cause of retinal degeneration [35-39]. Rewiring via new synaptic contacts between ganglion cells and remnant inner nuclear layers has been reported in rodents and humans with photoreceptor degeneration [38]. This new synaptic development may involve NMDA receptors.

Second, an immunohistological study using antibodies to NMDA receptors demonstrated strong expression of NMDA receptors in Müller cell processes in the inner retina [40]. NMDA receptors in many brain areas are reported to have considerable plasticity during development and in adulthood [41-43]. Further, a study showed that NMDA receptors were preserved to a greater extent in the superficial superior colliculus in aged dystrophic RCS rats than in WT rats, suggesting the possibility of an increased residual potential at the brain level when signal input is impaired because of photoreceptor loss [44]. These findings may indicate that NMDA receptors have the plasticity to change their expression in the visual pathway including the inner retina as photoreceptors degenerate in dystrophic RCS rats.

Conclusions

At the early stage of photoreceptor degeneration in dystrophic RCS rats, intravitreal NMDA injection attenuated OP amplitudes. This indicates that NMDA-sensitive neurons play a significant role in generating OP amplitudes in dystrophic rats. The early stage of photoreceptor degeneration may affect inner retinal function in dystrophic RCS rats.

References

1. Dowling JE, Sidman RL (1962) Inherited retinal dystrophy in the rat. J Cell Biol 14:73-109
2. 2.D'Cruz PM, Yasumura D, Weir J, Matthes MT, Abderrahim H, LaVail MM, Vollrath D (2000) Mutation of the receptor tyrosine kinase gene Mertk in the retinal dystrophic RCS rat. Human Mol Genet 9: 645-651
3. Duncan JL, LaVail MM, Yasumura D, Matthes MT, Yang H, Trautmann N, Chappelow AV, Feng W, Earp HS, Matsushima GK, Vollrath D (2003) An RCS-like retinal dystrophy phenotype in mer knockout mice. Invest Ophthalmology Vis Sci 44: 826-838
4. Feng W, Yasumura D, Matthes MT, LaVail MM, Vollrath D (2002) Mertk triggers uptake of photoreceptor outer segments during phagocytosis by cultured retinal pigment epithelial cells. J Biol Chem 277: 17016-17022
5. Vollrath D, Feng W, Duncan JL, Yasumura D, D'Cruz PM, Chappelow A, Matthes MT, Kay MA, LaVail MM (2001) Correction of the retinal dystrophy phenotype of the RCS rat by viral gene transfer of Mertk. Proc Natl Acad Sci USA 98: 12584-12589
6. Gal A, Li Y, Thompson DA, Weir J, Orth U, Jacobson SG, Apfelstedt-Sylla E, Vollrath D (2000) Mutations in MERTK, the human orthologue of the RCS rat retinal dystrophy gene, cause retinitis pigmentosa. Nature Genet 26: 270-271
7. McHenry CL, Liu Y, Feng W, Nair AR, Feathers KL, Ding X, Gal A, Vollrath D, Sieving PA, Thompson DA (2004) MERTK arginine-844-cysteine in a patient with severe rod-cone dystrophy: loss of mutant protein function in transfected cells. Invest Ophthalmol Vis Sci 45: 1456-1463
8. LaVail MM (2001) Legacy of the RCS rat: impact of a seminal study on retinal cell biology and retinal degenerative diseases. Prog Brain Res. 131: 617-627
9. Chader GJ (2002) Animal models in research on retinal degenerations: past progress and future hope. Vis Res 42: 393-399
10. Sieving PA, Frishman LJ, Steinberg RH (1986) Scotopic threshold response of proximal retina in cat. J Neurophysiol 56: 1049-1061
11. Viswanathan S, Frishman LJ, Robson JG, Harwerth RS, Smith EL 3rd (1999) The photopic negative response of the macaque electroretinogram: reduction by experimental glaucoma. Invest Ophthalmol Vis Sci. 40:1124-1136
12. Bush RA, Hawks KW, Sieving PA (1995) Preservation of inner retinal responses in the aged Royal College of Surgeons rat. Evidence against glutamate excitotoxicity in photoreceptor degeneration. Invest Ophthalmol Vis Sci 36: 2054-2062

13. Ohzeki T, Machida S, Takahashi T, Ohtaka K, Kurosaka D (2007) The effect of intravitreal *N*-methy aspartatic acid on the electroretinogram in Royal College of Surgeons rats. Jpn J Ophthalmol 51: 165-174
14. Machida S, Raz-Prag D, Fariss RN, Sieving PA, Bush RA (2008) Photopic ERG negative response from amacrine cell signaling in RCS rat retinal degeneration. Invest Ophthalmol Vis Sci 49: 442-452
15. Wachtmeister L (1998) Oscillatory potentials in theretina: what do they reveal. Prog Retin Eye Res 17: 485-521
16. Yonemura D, Aoki T, Tsuzuki K (1962) Electroretinogram in diabetic retinopathy. Arch Ophthalmol 68: 19-24
17. Shirao Y, Kawasaki K (1998) Electrical responses from diabetic retina. Prog Retin Eye Res 17: 59-76
18. Banin E, Cideciyan AV, Aleman TS, Petters RM, Wong F, Milam AH, Jacobson SG (1999) Retinal rod photoreceptor-specific gene mutation perturbs cone pathway development. Neuron 23: 549-557
19. Sakai T, Kondo M, Ueno S, Koyasu T, Komeima K, Terasaki H (2009) Supernormal ERG oscillatory potentials in transgenic rabbit with rhodopsin P347L mutation and retinal degeneration. Invest Ophthalmol Vis Sci 50: 4402-4409
20. Ikenoya K, Kondo M, Piao CH, Kachi S, Miyake Y, Terasaki H (2007) Preservation of macular oscillatory potentials in eyes of patients with retinitis pigmentosa and normal visual acuity. Invest Ophthalmol Vis Sci 48: 3312-3317
21. Slaughter MM, Miller RF (1983) The role of excitatory amino acid transmitters in the mudpuppy retina: an analysis with kainic acid and N-methyl aspartate. J Neurosci 3: 1701-1711
22. Akula JD, Mocko JA, Moskowitz A, Hansen RM, Fulton AB (2007) The oscillatory potentials of the dark-adapted electroretinogram in retinopathy of prematurity. Invest Ophthalmol Vis Sci 48: 5788-5797
23. Hood DC, Birch DG (1996-1997) Assessing abnormal rod photoreceptor activity with the a-wave of the electroretinogram: applications and methods. Doc Ophthalmol. 92: 253-267
24. Lamb TD, Pugh EN Jr (1992) A quantitative account of the activation steps involved in phototransduction in amphibian photoreceptors. J Physiol.449:719-758.
25. Zhang K, Yao G, Gao Y, Hofeldt KJ, Lei B (2007) Frequency spectrum and amplitude analysis of dark- and light-adapted oscillatory potentials in albino mouse, rat and rabbit. Doc Ophthalmol. 115:85-93.

26. Kizawa J, Machida S, Kobayashi T, Gotoh Y, Kurosaka D (2006) Changes of oscillatory potentials and photopic negative response in patients with early diabetic retinopathy. Jpn J Ophthalmol. 50: 367-373.
27. Takahashi T, Machida S, Masuda T, Mukaida Y, Tazawa Y(2005) Functional change with photoreceptor loss in rod and cone system in light-damaged rats. Curr Eye Res. 30: 703-713
28. Koyasu T, Kondo M, Miyata K, Ueno S, Miyata T, Nishizawa Y, Terasaki H(2008) Photopic electoretinograms of mGluR6-deficient mice. Curr Eye Res 33: 91-99
29. Hughes A (1979) A schematic eye for the rat. Vision Res 19: 569-588
30. Vaegan, Miller TJ (1994) Effect of kainic acid and NMDA on the pattern electroretinogram, the scotopic threshold response, the oscillatory potentials and the electroretinogram in the urethane anaesthetized cat. Vision Res 34: 1111-1125
31. Narahashi T, Moore JW, Scott WR (1964) Tetrodotoxin blockage of sodium conductance increase in lobster giant axons. J Gen Physiol 47: 965-974
32. Narahashi T (1974) Chemicals as tools in the study of excitable membranes. Physiol Rev 54: 813-889
33. Bloomfield SA (1996) Effect of spike blockade on the receptive-field size of amacrine and ganglion cells in the rabbit retina. J Neurophysiol 75: 1878-1893
34. Tanaka M, Machida S, Ohtaka K, Nitta J, Tazawa Y (2005) Third-order neuronal responses contribute to shaping the negative electroretinogram in sodium iodate-treated rats. Curr Eye Res 30: 443-453
35. Jansen HG, Sanyal S (1984) Development and degeneration of retina in rds mutant mice: electron microscopy. J Comp Neurol. 224: 71-84
36. Jansen HG, Sanyal S (1987) Synaptic changes in the terminals of rod photoreceptors of albino mice after partial visual cell loss induced by brief exposure to constant light. Cell Tissue Res 250: 43-52
37. Jansen HG, Sanyal S (1992) Synaptic plasticity in the rod terminals after partial photoreceptor cell loss in the heterozygous rds mutant mouse. J Comp Neurol 316: 117-125
38. Jones BW, Watt CB, Frederick JM, Baehr W, Chen CK, Levine EM, Milam AH, Lavail MM, Marc RE (2003) Retinal remodeling triggered by photoreceptor degenerations. J Comp Neurol 464: 1-16
39. Marc RE, Jones BW (2003) Retinal remodeling in inherited photoreceptor degenerations. Mol Neurobiol 28: 139-147
40. Grunder T, Kohler K, Guenther E (2001) Alterations in NMDA receptor expression during retinal degeneration in the RCS rat. Vis Neurosci 18: 781-787

41. Malenka RC, Nicoll RA (1993) NMDA-receptor-dependent synaptic plasticity: multiple forms and mechanisms. Trends Neurosci 16: 521-527
42. Collingridge GL, Bliss TV (1995) Memories of NMDA receptors and LTP. Trends Neurosci 18: 54-56
43. Constantine-Paton M (2000) The plastic brain. Neurobiol Dis 7: 515-519
44. Pothecary CA, Thompson H, Salt TE (2005) Changes in glutamate receptor function in synaptic input to the superficial superior colliculus (SSC) with aging and retinal degeneration in the Royal College of Surgeons (RCS) rat. Neurobiol Aging 26: 965-972

Figure legends

Figure 1: A representative scotopic ERG recorded from a WT rat at 30 days of age. The P3 was obtained by fitting the leading edge of the scotopic a-wave (dashed line). The P3 was then subtracted from the original waveform to obtain the P2 (A), which was digitally filtered between 65 and 500 Hz to extract the OPs. The amplitudes of OP1, OP2, OP3, and OP4 were measured and summed (B). ERG: electoretinogram, WT: wild-type

Figure 2: Age-related changes in ERGs of NMDA- (red symbols) and saline-injected eyes (black symbols) of WT (circle symbols) and dystrophic rats (square symbols). The averaged amplitudes of the a- (A) and b-waves (B) as well as ΣOPs (C) were plotted on a log scale against age. The average amplitude ratio of the ΣOPs/b-wave was plotted as a function of age (D). Error bars represent standard error of 5 animals at each time point. Asterisks indicate statistically significance by Bonferroni post hoc test following two-way ANOVA (*P<0.05, **P<0.01 and ***P<0.001). ERG: electoretinogram, NMDA: N-methyl-DL-aspartic acid, WT: wild-type

Figure 3: Representative ERG waveforms recorded from saline-injected (black lines) and NMDA-injected eyes (red lines) of WT and dystrophic rats at 25 and 30 days of age. ERG: electoretinogram, NMDA: N-methyl-DL-aspartic acid, WT: wild-type.

Figure 4: Averaged ΣOPs amplitudes of five 35-day-old animals were plotted against stimulus intensities to obtain intensity-response curves of NMDA- (red symbols) and saline-injected eyes (black symbols) of WT (circle symbols) and dystrophic rats (square symbols) (A). The ΣOPs sensitivity was defined as the stimulus intensity to elicit one-half of the maximum amplitude of the intensity-response curves (B). Error bars represent standard error of 5 animals at each stimulus intensity (A) or time point (B).

Figure 5: Isolated OPs recorded from saline-injected (black lines) and NMDA-injected eyes (red lines) of WT rats at 30 days of age. Implicit times were measured at peak of each wavelet (marked dashed line) prolonged by NMDA-injections (A). Implicit times were plotted against age for NMDA- (red symbols) and saline-injected eyes (black symbols) of WT (circle symbols) and dystrophic rats (square symbols) (B). Error bars represent the standard error of 5 animals at each time point.

Figure 6: FFT curves obtained from saline-injected (black lines) and NMDA-injected eyes (red lines) of WT and dystrophic rats (A). Peak frequencies were plotted against age for NMDA- (red symbols) and saline-injected eyes (black symbols) of WT (circle symbols) (B). AUCs of FFT curves were plotted on a log scale for saline- and NMDA-injected eyes of WT and dystrophic rats (C). Error bars represent standard error of 5 animals at each time point. FFT: fast Fourier transform, NMDA: N-methyl-DL-aspartic acid, WT: wild-type, AUC: area under the curve.

Figure 7: The ratios of variables in NMDA-injected eyes to those in saline-injected eyes (NMDA / saline) were calculated for ΣOP amplitudes (A) and AUCs (B and compared between WT (black bars) and dystrophic rats (white bars) at different ages. Error bars represent the standard error of 5 animals at each time point. Asterisks indicate statistically significance by Bonferroni post hoc test following two-way ANOVA (*P<0.05). N-methyl-DL-aspartic acid, WT: wild-type, AUC: area under the curve.