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Optokinetic test to evaluate visual acuity of each eye independently

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Abstract

A previously described optokinetic testing apparatus [Nat. Neurosci. 5 (2002) 53] was modified to measure vision in each eye separately for evaluation of monocular treatments. This apparatus consists also of a striped rotating drum. Ca. 170° of the drum are illuminated from outside and ca. 190° of the drum move behind a stationary black wall. The rat sits unrestrained in the drum center in a tube so that one eye is unexposed to the rotating stripes. Normal pigmented and retinal degenerate transgenic S334ter-3 rats were tested with the original and the modified apparatus. The usefulness of this method was tested in retinal degenerate rats with retinal transplants in one eye. In retinal degenerate animals, the amount of time (seconds) spent for head-tracking tended to be higher with the original method, possibly due to simultaneous stimulation of both eyes. In rats with retinal transplants, visual responses were significantly preserved in transplanted eyes at late stages of retinal degeneration. In conclusion, contributions from the fellow eye to the optokinetic tracking response can be limited by this testing modification, which is useful for evaluation of treatment effects to one eye.

Keywords: Optokinetic response; Head-tracking; Visual behavior; Retinal degeneration

1. Introduction

Functional assessment of visual responses is important for evaluating various treatments in animal models of retinal degeneration. This may be done by electrophysiology and/or visual behavior testing (Coffey et al., 2002; Lawrence et al., 2000; Lund et al., 2001a,b; Sagdullaev et al., 2003; Woch et al., 2001). Behavioral visual acuity tests are conducted to demonstrate visual responses mediated through the central neural circuitry. Various kinds of behavioral tests are employed in rodents to evaluate progression of visual loss consequent to retinal degeneration, and to assess the functional effects of various therapeutic interventions (Lund et al., 2001a). These tests include simple startle reflex (del Cerro et al., 1995) and orientation tests (Hetherington et al., 2000), as well as more complex light discrimination and maze tests (Coffey et al., 2002; Kwan et al., 1999; Little et al., 1998; Prusky et al., 2000).

Another particularly effective test of visual performance measures an animal's ability to track moving stimuli. This head-tracking (HT) test is based on the optokinetic response and was originally described by Cowey and Franzini (1979). The optokinetic response is a compensatory eye movement that takes place in the direction of the movement of a stimulus. It helps to reduce the movement of the images of the external world across the retina. An animal with normal vision automatically tracks moving stimuli by turning its head in the direction of the movement. At a certain point, it will turn the head back and will start tracking over again. By scoring the total time spent tracking the movements, it is possible to establish a measure of visual function (Coffey et al., 2002; Lund et al., 2001a). The stimulus, commonly a high contrast alternating stripe pattern, is usually presented using a rotating drum. By varying the stripe width (different

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grating frequencies), a measure of visual acuity can be established.

Recently, a head-tracking test has been employed to demonstrate the degree of visual loss in transgenic mice (Thaung et al., 2002) and the improvement of visual behavior in RCS rats after retinal pigment epithelium (RPE) cell transplantation (Coffey et al., 2002; Lund et al., 2001a). However, this technique is not reliable in albino rats due to their abnormal visual sensory apparatus (Precht and Cazin, 1979). Vestibular nuclear neurons of the horizontal canal system of albino rats fail to respond to optokinetic stimulation, and an optokinetic nystagmus cannot be elicited.

Mammals whose optokinetic responses have been studied extensively are divided into two major classes on the basis of their visual field. The so called 'higher' mammals, which possess considerable overlap of visual field and good binocular vision, and 'lower' mammals, whose eyes are laterally placed with little resultant binocular vision. In 'higher' mammals, a group, which includes felines and primates, during monocular optokinetic stimulation, the responses are very similar to stimulation in both directions of rotation. The visual cortex has been suggested to play a major role in maintaining this symmetry between nasotemporal and temporonasal stimulation (Flandrin et al., 1992; Tusa et al., 1989; Zee et al., 1987). Of note, among human and primate infants, the asymmetry in optokinetic response observed during nasotemporal versus temporonasal stimulation is believed to be due to their immature cortical system (Fu and Boothe, 2001). In rabbits (an example of 'lower' mammals with minimal binocular vision), nasotemporal stimulation is rather ineffective in eliciting the optokinetic response (Hobbelen and Collewijn, 1971). Among pigmented rats, the gain of the optokinetic response to monocular temporonasal versus nasotemporal stimulation is highly asymmetric (Harvey et al., 1997).

Optokinetic response testing with the original head-tracking apparatus described by Coffey and coworkers (Coffey et al., 2002; Lawrence et al., 2000; Lund et al., 2001a,b) allows free movement of the rats. Furthermore, both eyes are exposed to the optokinetic stimulus with the presumption that each eye responds primarily to movement in the temporonasal direction. The contribution of both eyes to the response, however, cannot be ruled out. We modified the original apparatus to allow responses from each eye to be measured individually, thereby enabling a direct comparison of the head-tracking response between the left and right eyes during temporonasal and nasotemporal stimulation. Comparison of results obtained with the original and modified apparatus in both normal and retinal degenerate rats are described in this report. This study used pigmented S334ter-line-3 rats with fast retinal degeneration that lose all rods by 3–4 weeks postnatally because they express a mutant human rhodopsin (Liu et al., 1999; Sagdullaev et al., 2003). The usefulness of the modified apparatus was further evaluated in pilot studies to investigate whether retinal transplants to one eye have a beneficial effect in rats with retinal degeneration.

2. Materials and methods

2.1. Experimental animals

Animals were maintained in accordance with the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmologic and Vision Research, and institutional approval was obtained. Normal pigmented Long Evans rats (120-day old) and transgenic pigmented S334ter-line-3 retinal degenerate rats (135- and 205-day old) expressing a mutated human rhodopsin protein (Liu et al., 1999; Sagdullaev et al., 2003) were tested. Founder breeding pairs of the transgenic rats were produced by Chrysalis DNX Transgenic Sciences, Princeton, NJ, and kindly provided by Dr. M.M. LaVail, UCSF, San Francisco, CA. Rats of either sex were used.

2.2. Original optokinetic apparatus

The original rodent optokinetic testing apparatus was designed according to the specifications previously described by Lund and Coffey (Coffey et al., 2002; Lawrence et al., 2000; Lund et al., 2001a,b). The original device consists of a rotating drum with alternating high-contrast stripes (black and white) of different spatial frequencies (0.125, 0.25, and 0.5 cycles per degree), which is illuminated from above (250 cd/m^2). The rat is placed in a clear plastic stationary round chamber at the center of the drum, which allows visualization and exposure of both eyes to the stimulus. The observer waits for the animal to settle in the chamber before initiating drum rotation.

2.3. Monocular optokinetic apparatus

The modified apparatus (Fig. 1) also consists of a rotating drum with stripes of the above three different spatial frequencies. Ca. 170° of the rotating drum are evenly illuminated with three flood lights (250 cd/m^2 at the level of the rat's eye) from the outside. The light intensity can be regulated with a dimmer. Ca. 190° of the drum move behind a stationary black wall opposite from the light path so that only one eye is exposed to the rotating stripes. The rat is placed inside a plastic tube attached to the top of a 9 in. high holder. Different size tubes are used adapted to the size to the rat. The holding tube restricts movements of the rat's body, but allows free, unrestricted movement of the head by openings at the front sides. To prevent the rat from leaving the tube, a mild electrical shock plate is placed just outside. The rats typically only need a single shock to always remember not try to climb out. The holding tube can easily be turned 180°, for subsequent exposure of the fellow eye to

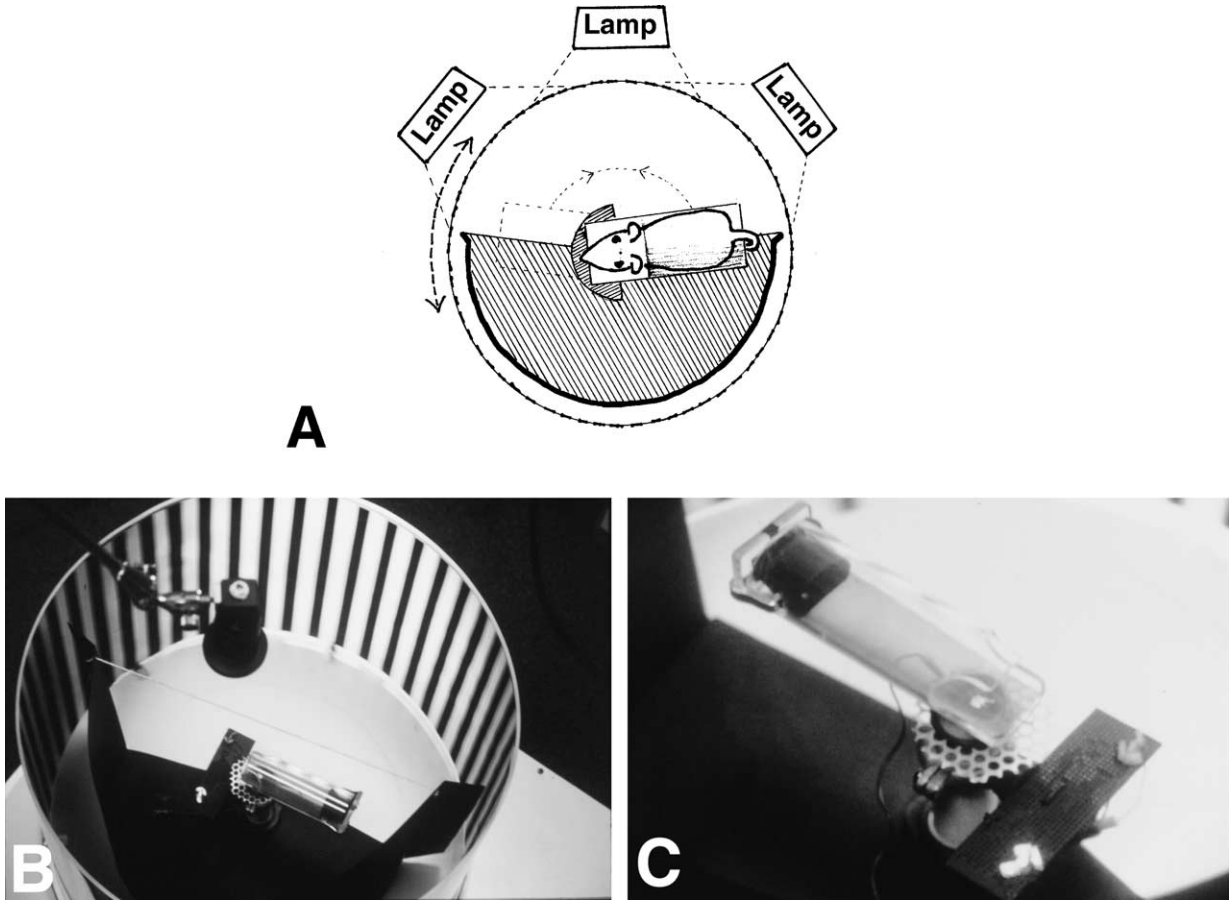

Fig. 1. Behavioral testing apparatus: (A) schematic drawing. The modified apparatus consists of a rotating drum with stripes. Ca. 170° of the drum are evenly illuminated from the outside and ca. 190° of the drum move behind a stationary black wall, which blocks the path from the light source. (B) Photograph of the drum from above, showing the video camera that records the head movements, the stationary black wall, and the rat holder in the center. (C) Rat holder: the rat is placed into a narrow tube (different sizes of tubes depending on the rat size) which can be turned 180°. The front of the tube has open sides for the head. An electrically charged plate prevents the rat from climbing out. Once exposed to the shock, the rat will always sit calmly, turning the head only. The rats are tested for 4 min during one session, 2 min for each eye, 1 min in each direction of the striped drum. The time (in seconds) spent turning the head following the rotation of the drum is recorded as 'head-tracking'. Two different stripes widths correspond to two grating frequencies of 0.25 cycles per degree (1 cm, medium stripes), and 0.125 cycles per degree (2 cm, large stripes), with a constant rotational speed of two turns per minute of the drum. Only pigmented rats can be tested by this method (see Section 1).

the optokinetic stimulus. A video camera records the rat's head movements for later analysis and scoring.

2.4. Optokinetic testing protocol

Using each device (original and modified), 120-day old normal pigmented Long Evans rats and 135- and 205-day old transgenic pigmented S334ter-line-3 retinal degenerate rats expressing a mutated human rhodopsin protein (Sagdullaev et al., 2003) were tested. For each rat, the drum was rotated (at a constant rate of 12°/s) for 120 s per eye, 60 s clockwise and 60 s counterclockwise. The efficacy of the modified apparatus in monocular visual assessment was evaluated using pigmented S334ter-line-3 retinal degenerate rats that received retinal transplants in one eye at the age of 16–27 day. The details of the transplantation surgical procedure is described elsewhere (Sagdullaev et al., 2003; Woch et al., 2001). Animals were tested weekly from 130 to 240 days of age. For a direct comparison of transplanted and non-transplanted eye, the total head-tracking score (both temporonasal and nasotemporal) for each eye was computed. All animals evaluated in this study had already been accustomed to the optokinetic apparatus. Tests were conducted in random order, with some animals being tested first with the original apparatus and other animals tested first with the modified apparatus. An interval of 1 week was passed between the two tests to avoid the possible development of habituated behavior. A head turn was scored only when the speed of tracking corresponded to the speed of the rotation of the stripes. Habitual and other random head movements were excluded when computing the score, which was defined as the total amount of time (in seconds) spent head-tracking for each eye during the 120 s testing period. A spatial frequency of 0.125 cycles per degree, the stripe width, which provided the most robust head-tracking response during preliminary studies,

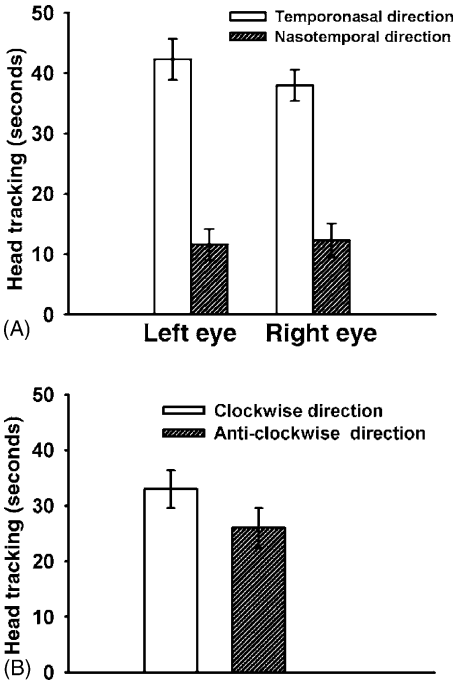

Fig. 2. Optokinetic testing of normal pigmented rats. Comparison of the modified method (A) with Coffey method (B). In the modified method, the head-tracking score in the temporonasal direction is comparable to the score in the Coffey method. The nasotemporal score in the modified method is very much reduced.

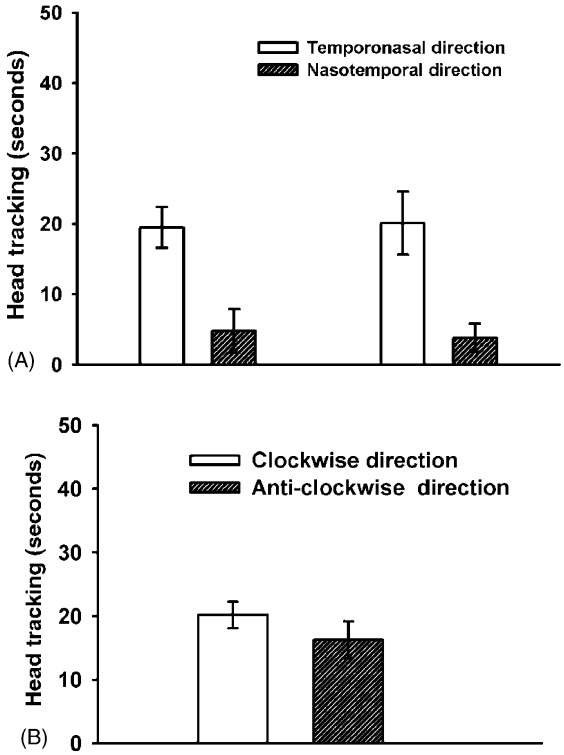

Fig. 3. Optokinetic testing of 135-day old S334ter-line-3 rats. Comparison of the modified method (A) with Coffey method (B). In the modified method, the head-tracking score in the temporonasal direction is comparable to the Coffey method. In the modified method, the temporonasal score is reduced by ca. 50% compared to normal rats (Fig. 1). The reduction is not so pronounced with the Coffey method.

was used for all experiments in this report. During pilot experiments, the reliability of the scoring system was confirmed by reevaluation of the video recordings by a second masked grader.

Statistical comparisons (paired *t*-test, two tailed) were performed, using a statistics package of GraphPad Software Inc., San Diego, CA.

3. Results

During monocular optokinetic stimulation testing using the modified device, a highly asymmetrical head-tracking score ($P < 0.001$) was observed when comparing responses to the rotation of the drum in the nasotemporal versus the much stronger response to the temporonasal direction (Figs. 2–4). In contrast, the difference was significantly less pronounced when comparing clockwise versus counterclockwise directions of drum rotation using the original apparatus. With the modified setup, the head-tracking score of the mainly monocular nasotemporal stimulation was significantly lower than the head-tracking score of binocular testing (clockwise and counterclockwise drum rotation) using the original device (Figs. 2–4). In contrast, the head-tracking score of temporonasal stimulation using the modified apparatus for each eye was similar to the head-tracking score for clockwise (or counterclockwise) rotation using the original device in normal pigmented Long Evans rats.

The two methods were also evaluated with pigmented rhodopsin-mutant rats (S334ter-line-3) at an early and late stage of photoreceptor degeneration. When tested at the earlier age (135 days), a considerable level of visual function and robust head-tracking response was found to persist in these rats (Fig. 3). Interestingly, at this age, the head-tracking score was the same for both the modified and original devices (both for the clockwise and counterclockwise direction of drum rotation).

At a later stage of degeneration of S334ter-line-3 rats (age 205 days), when using the modified device, the head-tracking score of the temporonasal stimulation was reduced (Fig. 4). A higher head-tracking score was observed when the same animals were tested using the original apparatus (left eye, $P < 0.07$ and right eye, $P < 0.2$, Fig. 5).

The usefulness of monocular visual assessment was tested using retinal degeneration rats that had received retinal transplants in one eye. Among non-transplanted S334ter-3 rats, good visual head-tracking score persists up to about 5 months of age (data not shown). After 166 days of age, a progressive loss of the head-tracking score was observed and after 180 days, the visual sensitivity decreased faster in both eyes (Fig. 5B). At 240 days of age, only a very low head-tracking score could be observed in non-transplanted rats. The head-tracking score for transplanted eyes remained

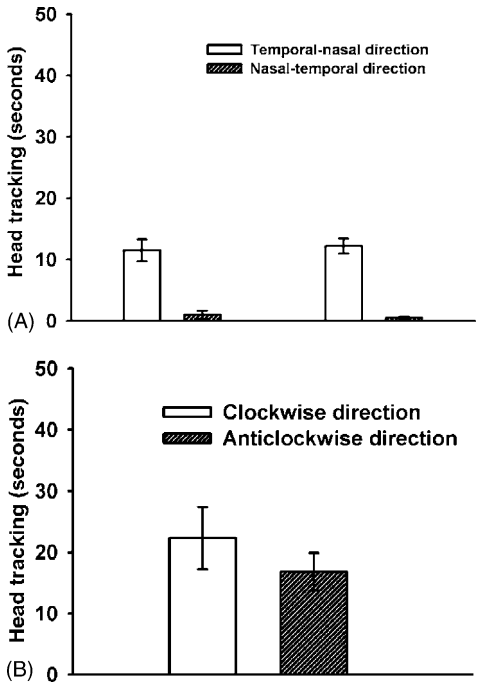

Fig. 4. Optokinetic testing of 205-day old S334ter-line-3 rats. Comparison of the modified method (A) with Coffey method (B). In the modified method, the head-tracking score in the temporal-nasal direction is reduced by ca. 50% compared to the Coffey method. The nasotemporal score in the modified method is now almost zero whereas there is no significant difference between clockwise and counterclockwise directions in the Coffey method.

significantly higher compared to the control eyes until the end of testing period (Fig. 5A). In all the control rats without retinal transplants, there was no apparent difference in the temporal progression of visual head-tracking loss between the right and left eye (Fig. 5B).

4. Discussion

The modified optokinetic apparatus has several advantages compared to other testing methods: it is simple and provides a lot of data in a short testing session. The modifications to the original optokinetic head-tracking apparatus described in this report allow better monocular visual behavioral testing in rats. This modification can be particularly important, when only one eye of an animal is subjected to an experimental intervention and precise comparison between the treated and untreated eyes of the rat is required.

Optokinetic testing with the modified apparatus demonstrates that during monocular visual behavioral testing, the head-tracking scores for nasotemporal and temporonasal stimulation are highly asymmetric. It has been previously reported that during monocular optokinetic stimulation in rodents, the response to temporonasal stimulation is much more robust than the response following nasotemporal stimulation (Harvey et al., 1997; Hobbelen and Collewijn, 1971). Interestingly, using the original device, when both eyes are simultaneously exposed to the stimulus (one eye is stimulated in a nasotemporal direction and the other is stimulated temporonasally) the degree of asymmetry (counterclockwise versus clockwise rotation) is attenuated in all the rats tested. Although we presume that the observed head-tracking response is mostly derived or driven by the eye receiving the temporonasal stimulation, it is not a true monocular test. A contribution from the fellow eye cannot be ruled out. Such a 'fellow eye' response needs to be considered in the evaluation of monocular experimental interventions. However, our test is not purely monocular because the other eye could not be covered up. It may be possible to train the rat to accept a temporary eye cover, but this would complicate the testing setup and require more time.

The rat holding chamber used in the original apparatus provides considerable freedom of movement for the rat. If the rat moves, the distances to the drum stripes and thus the apparent rotation speed will be slightly different for each eye so that there will be small variations in the results. In addition, regardless of the direction of drum rotation, tracking in the preferred direction (temporonasal) is happening with either one of the eyes. This again poses problems when the goal is to compare visual function between the two eyes of a given animal. This is especially important, given that numerous animal studies evaluating therapeutic interventions for retinal disease, use only one eye as the treatment eye, and maintain the fellow eye as a control (Coffey et al., 2002; Kwan et al., 1999; Lawrence et al., 2000; Little et al., 1998; Lund et al., 2001b; Sagdullaev et al., 2003; Whiteley et al., 2001; Woch et al., 2001).

No significant differences in head-tracking scores between the original and modified devices were observed when the above experiments were conducted at an early age of retinal degeneration. Interestingly, at a later age (205 days), the head-tracking score was higher when the original setup was used. Thus, the fellow eye contribution may be of greatest importance and significance in animals with more advanced degeneration (which are often the target animals of various monocular therapeutic interventions).

The advantage of the modified visual head-tracking apparatus was more apparent when testing the rats for transplanted versus non-transplanted eye. The modified apparatus clearly demonstrated the preservation of visual responses by retinal transplantation. Although the progressive deterioration of head-tracking behavior was observed among all the retinal degeneration rats, the transplanted rats performed significantly better than the non-transplanted control retinal degeneration rats. This is consistent with the recent electrophysiological findings in rat models of retinal degeneration (Sagdullaev et al., 2003; Woch et al., 2001).

The monocular optokinetic testing demonstrated that among pigmented S334ter-line-3 rats, the progression of visual loss during the process of takes place symmetrically in both eyes. This observation is consistent with the previ-

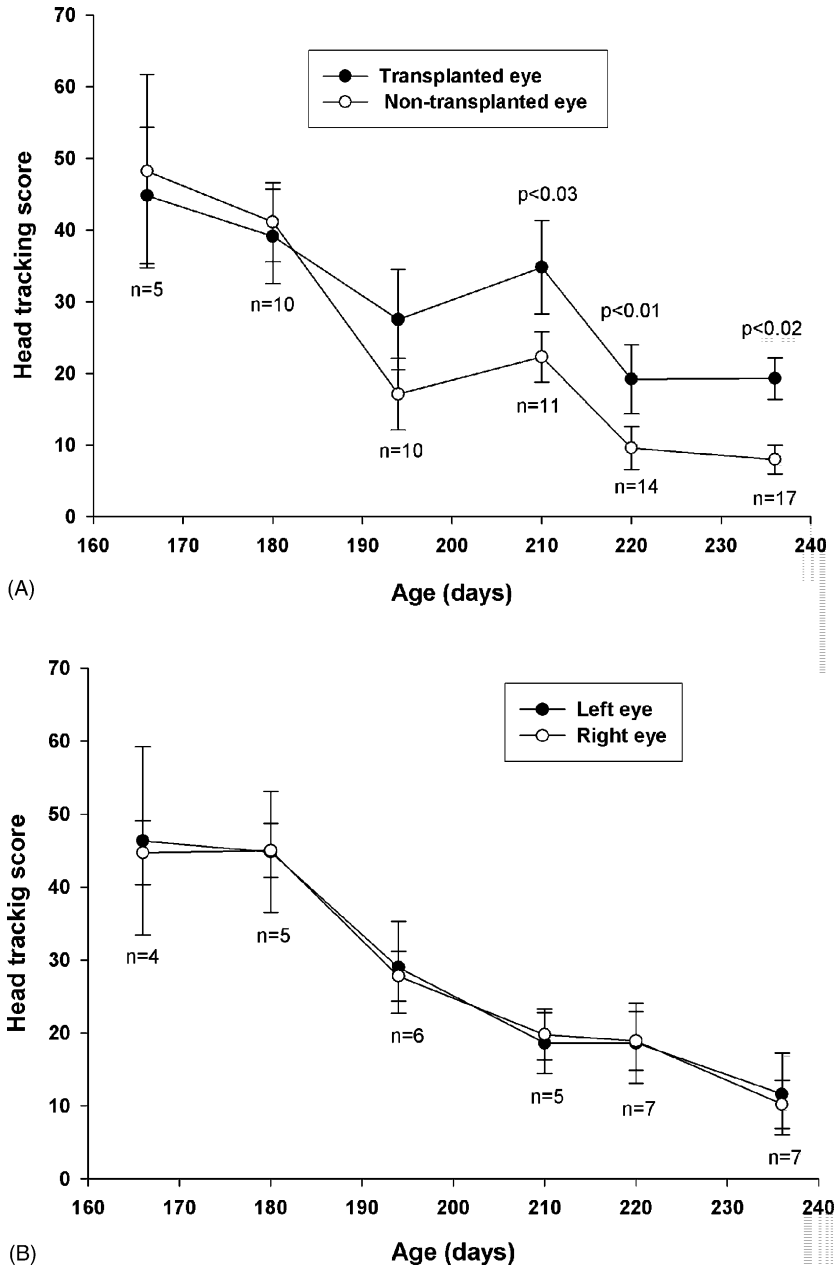

Fig. 5. Optokinetic testing of S334ter-line-3 rats, 166–236 day old, modified method. The head-tracking score is compared between left and right eye among transplanted (A) and age-matched no surgery (B) S334ter-3 rats. Significant preservation of visual responses was seen in the transplanted eyes at later stages of retinal degeneration. Among non-transplanted rats the progression of visual loss is more symmetrical in both eyes.

ous electrophysiological findings in these rats (Sagdullaev et al., 2003).

In summary, the modified optokinetic visual behavioral testing apparatus described in this report has important features. First, it will allow better monocular vision assessment. Monocular vision assessment appears to be particularly useful in the advanced stages of retinal degenerations, and can be of value in the evaluation of various monocular therapeutic interventions. Second, it is a very simple and fast testing procedure, which can be repeated many times over during a long time period in the same animal.

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References

Coffey PJ, Girman S, Wang SM, Hetherington L, Keegan DJ, Adamson P, et al. Long-term preservation of cortically dependent visual function in RCS rats by transplantation. Nat Neurosci 2002;5:53–6.

Cowey A, Franzini C. The retinal origin of uncrossed optic nerve fibers in rats and their role in visual discrimination. Exp Brain Res 1979;35(3):443–56.

del Cerro M, DiLoreto Jr D, Cox C, Lazar ES, Grover DA, del Cerro C. Neither intraocular grafts of retinal cell homogenates nor live non-retinal neurons produce behavioral recovery in rats with light-damaged retinas. Cell Transplant 1995;4:133–9.

Flandrin JM, Courjon JH, Orban GA, Sprague JM. Long-term impairment of cat optokinetic nystagmus following visual cortical lesions. Exp Brain Res 1992;88:594–608.

Fu LN, Boothe RG. A psychophysical measurement and analysis of motion perception in normal and binocularly deprived monkeys. Invest Ophthalmol Vis Sci 2001;42:2547–53.

Harvey RJ, De'Sperati C, Strata P. The early phase of horizontal optokinetic responses in the pigmented rat and the effects of lesions of the visual cortex. Vision Res 1997;37:1615–25.

Hetherington L, Benn M, Coffey PJ, Lund RD. Sensory capacity of the Royal College of Surgeons rat. Invest Ophthalmol Vis Sci 2000;41:3979–83.

Hobbelen J, Collewijn H. Effect of cerebro cortical and collicular ablations upon the optokinetic reactions in the rabbit. Doc Ophthalmol 1971;30:227–36.

Kwan AS, Wang S, Lund RD. Photoreceptor layer reconstruction in a rodent model of retinal degeneration. Exp Neurol 1999;159:21–33.

Lawrence JM, Sauve Y, Keegan DJ, Coffey PJ, Hetherington L, Girman S, et al. Schwann cell grafting into the retina of the dystrophic RCS rat limits functional deterioration. Invest Ophthalmol Vis Sci 2000;41:518–28.

Little CW, Cox C, Wyatt J, del Cerro C, del Cerro M. Correlates of photoreceptor rescue by transplantation of human fetal RPE in the RCS rat. Exp Neurol 1998;149:151–60.

Liu C, Li Y, Peng M, Laties AM, Wen R. Activation of caspase-3 in the retina of transgenic rats with the rhodopsin mutation s334ter during photoreceptor degeneration. J Neurosci 1999;19:4778–85.

Lund RD, Adamson P, Sauve Y, Keegan DJ, Girman SV, Wang S, et al. Subretinal transplantation of genetically modified human cell lines attenuates loss of visual function in dystrophic rats. Proc Natl Acad Sci USA 2001b;98:9942–7.

Lund RD, Kwan AS, Keegan DJ, Sauve Y, Coffey PJ, Lawrence JM. Cell transplantation as a treatment for retinal disease. Prog Retin Eye Res 2001a;20:415–49.

Precht W, Cazin L. Functional deficits in the optokinetic system of albino rats. Exp Brain Res 1979;37(1):183–6.

Prusky GT, West PW, Douglas RM. Behavioral assessment of visual acuity in mice and rats. Vision Res 2000;40:2201–9.

Sagdullaev BT, Aramant RB, Seiler MJ, Woch G, McCall MA. Retinal transplantation-induced recovery of retinotectal visual function in a rodent model of retinitis pigmentosa. Invest Ophthalmol Vis Sci 2003;44:1686–95.

Thaung C, Arnold K, Jackson IJ, Coffey PJ. Presence of visual head tracking differentiates normal sighted from retinal degenerate mice. Neurosci Lett 2002;325:21–4.

Tusa RJ, Demer JL, Herdman SJ. Cortical areas involved in OKN and VOR in cats: cortical lesions. J Neurosci 1989;9:1163–78.

Whiteley SJ, Klassen H, Coffey PJ, Young MJ. Photoreceptor rescue after low-dose intravitreal IL-1beta injection in the RCS rat. Exp Eye Res 2001;73:557–68.

Woch G, Aramant RB, Seiler MJ, Sagdullaev BT, McCall MA. Retinal transplants restore visually evoked responses in rats with photoreceptor degeneration. Invest Ophthalmol Vis Sci 2001;42:1669–76.

Zee DS, Tusa RJ, Herdman SJ, Butler PH, Gucer G. Effects of occipital lobectomy upon eye movements in primate. J Neurophysiol 1987;58:883–907.