Electroretinographic Responses to Alternating Gratings Before and After Section of the Optic Nerve

Abstract. *Electroretinographic (ERG) responses to sinusoidal gratings reversed in contrast (pattern-reversal ERG) were recorded from both eyes of cats before and after unilateral section of the optic nerve. In the eye ipsilateral to the section, the pattern-reversal ERG remained unaltered for a few days after the section, then progressively decreased in amplitude, first at low and then at high spatial frequencies, to disappear completely about 4 months after the section, when ganglion cell degeneration was practically complete. The flash ERG remained unaltered. No alteration was observed in the contralateral eye.*

It has long been known that the vertebrate electroretinogram (ERG) in response to flashes of light consists of various components that have been ascribed to electrical sources located mainly in the receptor layer and in the inner nuclear layer of the retina (*1*, *2*). The contribution of ganglion cells to the flash ERG is regarded as null or negligible (*1*-*3*).

Diffuse light is not a very effective stimulus for neurons having a receptive field organized in antagonistic regions, although it is a powerful stimulus for retinal receptors and other neurons in the external layers of the retina. For retinal ganglion cells, contrast reversal of periodical patterns, like gratings with no temporal change in mean luminance, is a much more effective stimulus than unpatterned light. One may expect, therefore, that the ERG responses to alternating gratings (*4*) show some sign of the activity of ganglion cells. This point can be investigated by recording pattern-reversal ERG in the animal before and after section of the optic nerve, which causes retrograde degeneration of ganglion cells. The present experiments on the cat provide evidence that no measurable ERG response to alternating gratings can be recorded after ganglion cell degeneration.

Experiments were performed in eight adult cats. An endotracheal tube and a venous cannula were inserted under halothane anesthesia. The animal was then mounted in a stereotaxic apparatus and paralyzed with Pavulon. Artificial ventilation was supplied with a mixture N_2O (75 percent) + O_2 (25 percent). An electrocardiogram was continuously monitored, and body temperature was maintained at 38°C. Pupils were dilated with atropine. Platinum ring electrodes for ERG recording were applied to the corneas with contact lenses having an artificial pupil 4 mm in diameter. Refraction was corrected with additional lenses (usually +2 to +4 diopters). ERG's were recorded alternatively from either eye while the nontested eye was patched.

The papillae of both eyes were projected by inverted ophthalmoscopy on a tangent screen 40 cm from the eye. The screen was then replaced by a display (Hewlett-Packard 1300A) (20 by 25 cm) positioned (at 40 cm) in such a way as to cover the central part of the visual field of either eye. Sinusoidal gratings of various spatial frequencies were generated on the display and shifted in spatial phase by 180° (pattern reversal) at the rate of 8 Hz (16 reversals per second). In some cats, lower rates of alternations (2 and 4 Hz) were also used. The contrast was 30 percent, and the mean luminance was 10 cd/m². The same display was used to present homogeneous (unpatterned) flickering lights (on-off luminance modulation at various rates) and light flashes. In preliminary experiments, ERG's were recorded in response to gratings of 0.17 to 2.5 cycle/deg and to flickering lights.

The responses were filtered with a band-pass filter (slope, 6 dB per octave) between 6 and 60 Hz for gratings alternating at 8 Hz and between 0.6 and 60 Hz for light flashes or gratings at lower rates. The ERG's were conventionally amplified, fed into an averaging computer, and recorded by an x-y plotter. Usually at least 500 responses were averaged in order to sufficiently improve the signal-to-noise ratio. At the end of the recording session, the cat was allowed to recover from paralysis. A few days after the preliminary experiment, the cat was anesthetized with pentobarbital (35 mg per kilogram of body weight) and the right optic nerve sectioned by an oral approach before the chiasm. The first recording session was performed in two cats on the day of the operation, in one cat 6 days later, and in the other cats 8 days to 3 weeks later. The ERG's were recorded from each cat at various times during the first 4 months after the operation. In the first session after optic nerve section, pattern-reversal cortical evoked potentials were also recorded with monocular stimulus presentation to make sure that no cortical response was present from the right eye. After the last recording session, the cat was anesthetized with pentobarbital, killed, and perfused with 10 percent Formol for subsequent control of the section and for retinal histology.

The ERG in response to gratings alternating in phase at 8 Hz, when band-pass filtered, has an approximately sinusoidal

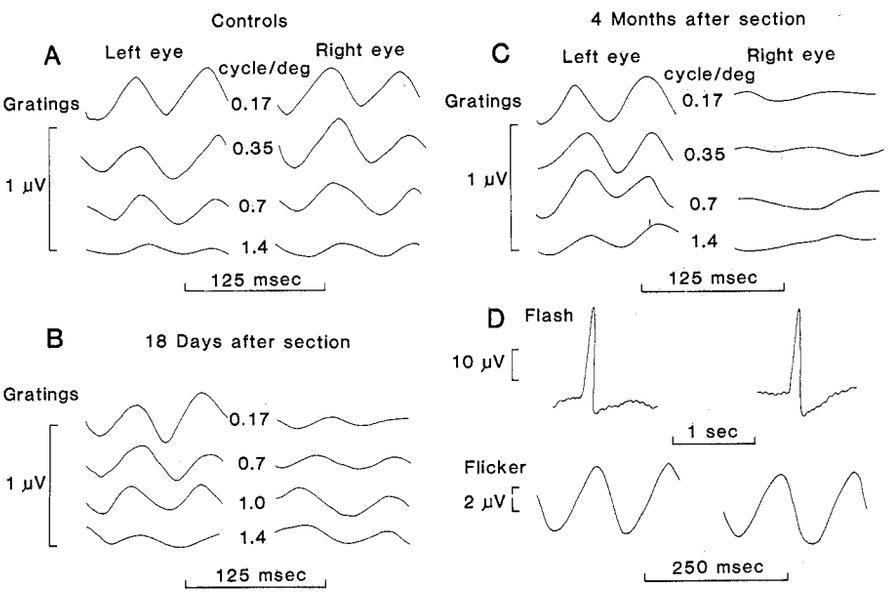

Fig. 1. Examples of pattern-reversal ERG's recorded from one cat before and after the section of the right optic nerve. (A) Control records obtained from the two eyes before the section of the optic nerve. (B and C) Records obtained 18 days and 4 months after the optic nerve section. Each record is the average of 500 responses. Stimulus: vertical sinusoidal grating reversed in contrast 16 times per second; contrast, 30 percent; mean luminance, 10 cd/m². The spatial frequency is indicated next to each record. (D) ERG in response to 50-msec light flashes (250 trolands) and to light flickering at 8 Hz (mean luminance, 10 cd/m²; amplitude of square-wave modulation, 40 percent) recorded from the two eyes 4 months after section of the right optic nerve.

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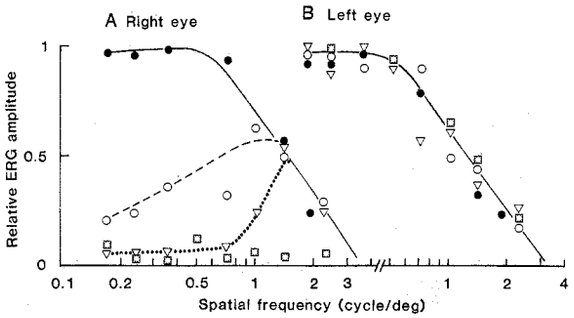

Fig. 2. Relative amplitude of the pattern-reversal ERG plotted as a function of spatial frequency obtained from one cat for the eye ipsilateral to the optic nerve section (A) and the contralateral eye (B). Stimulus conditions were as for data in Fig. 1. Data obtained in four experimental sessions: control session, before optic nerve section (●); session at 18 days (○); 70 days (▽) and 4 months (□) after optic nerve section. Each point is the average of two to four records.

waveform (*5*) with a temporal frequency of 16 Hz, corresponding to the second harmonic of the stimulus frequency (see time scale in Fig. 1, A to C). This property is peculiar to the response to phase-alternating gratings. The response to unpatterned light flickering at 8 Hz, when band-pass filtered, also has a sinusoidal waveform, but has a temporal frequency of 8 Hz (Fig. 1D). In addition, the response to flickering light is comparatively much greater in amplitude.

Examples of control ERG's recorded before the optic nerve was sectioned, are shown in Fig. 1A. The ERG amplitude is independent of spatial frequencies from 0.7 to 0.5 cycle/deg and then progressively decreases (Fig. 1A and Fig. 2, filled symbols). The highest spatial frequency at which a pattern-reversal ERG could be obtained was 2.5 cycle/deg. The responses usually had the same amplitudes in the two eyes (Fig. 1A and Fig. 2). If the ERG amplitudes in the high-frequency range are fitted with a straight line, extrapolation of this line to zero amplitude meets the log spatial frequency axis between 3 and 4 cycle/deg (Fig. 2, solid lines), which is not much below the behavioral visual acuity of cats at these luminance levels (*6*).

The pattern-reversal ERG responses recorded either soon after the optic nerve section or 6 days later had the same amplitude in the two eyes and were comparable to the control ERG's recorded before the operation. This was taken as evidence that the operation did not cause circulatory disturbances or other secondary effects that could impair the ERG responses (*7*).

The earliest unequivocal effects of optic nerve section on the pattern-reversal ERG of the ipsilateral eye were observed in one cat 18 days after the operation (Fig. 1) and in the other cats between the fourth and sixth week (*8*). These effects consisted of a considerable reduction in the amplitude of the ERG responses at low spatial frequencies (Fig. 1B and Fig. 2A, open circles) while the ERG's at or above 1 cycle/deg were unaffected. No clear response to the lowest spatial frequencies could be obtained by about 2 months later. Even the ERG's at 1 cycle/deg were attenuated (Fig. 2A, open triangles).

Four months after the operation, we could not obtain a pattern-reversal ERG from the right eye at any spatial frequency either at 8 Hz (Fig. 1C and Fig. 2A, open squares) or at lower rates of alternation. These findings were repeated in seven cats. In one cat, the pattern-reversal ERG from the right eye 40 days after the operation was still comparable to that of the controls. At the autopsy, the section of the right optic nerve was found to be incomplete.

In all cats, the ERG in response to light flashes or flicker remained normal and had comparable amplitude in both eyes throughout the 4-month period following the operation (Fig. 1D) (*9, 10*).

In agreement with what has previously been reported (*11*), the whole-mounted retinas of our animals showed degeneration of the ganglion cell layer 4 months after the optic nerve section. The degeneration was as pronounced in the periphery as in the center of the retina. According to previous reports (*11*), there is no clear sign of ganglion cell degeneration in preparations obtained earlier than 3 months after the section. The disappearance of the pattern-reversal ERG 4 months after the section is therefore likely to result from ganglion cell degeneration.

It is tempting to conclude that ganglion cell activity is the main source of the pattern-reversal ERG. Obviously, we cannot exclude the possibility that the degeneration of the ganglion cells may have secondary effects capable of influencing other possible generators of the ERG, although this is rather unlikely since the flicker ERG remains totally unaffected even after complete degeneration of the ganglion cells.

The ERG is a mass response, and it is likely that cells with receptive fields of different sizes contribute with different weights to the ERG potentials in different bands of the spatial frequency spectrum. In particular, the ERG response to the higher spatial frequencies is likely to reflect the activity of retinal neurons with finer receptive fields and higher-frequency cutoff, whereas neurons with broad receptive fields and low spatial frequency cutoff contribute to the ERG only in the low spatial frequency range. The fact that, after section of the optic nerve, ERG responses remain longer unattenuated at high than at low spatial frequencies could therefore indicate that ganglion cells with small receptive fields that are most frequent in the central retina are impaired later than cells with larger receptive fields. The majority of ganglion cells with small receptive fields that can respond to gratings of high spatial frequency (*12*) are known to be of the X type, with slow conducting nerve fibers. We could expect, therefore, that signs of anatomical degeneration should appear first for other types of cells than for those. No direct histological evidence is available to support or disprove this hypothesis, but we are currently planning to examine histologically preparations at various stages after the optic nerve section.

So far as our results can be extended to the human retina, we may expect that in patients with ganglion cell degeneration resulting either from compression of the optic nerve or retina, or from chronic optic nerve neuritis, the pattern-reversal ERG should be absent or abnormal. Pattern-reversal ERG's have yet to be measured, but in the majority of these patients the ERG response to light flashes gives no sign of abnormality (*10*).

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References and Notes

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7. This finding also rules out hypothetical effects of centrifugal fibers, which, however, have not been demonstrated to exist in the optic nerve of the cat [for example (*2*), p. 554)].