The Impact of Basic Fibroblast Growth Factor on Photoreceptor Function and Morphology

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Purpose. To assess the impact of basic fibroblast growth factor (bFGF) on photoreceptor function and morphology.

Methods. Impact was assessed in two models. In one, the endogenous expression of bFGF in photoreceptors was raised by sectioning one optic nerve of rats 3 to 4 weeks before study. In the other, bFGF was injected into the vitreous chamber in rats and cats. Retinal function was assessed from the electroretinogram (ERG), and retinal morphology was studied using DNA dyes, immunolabeling, and in situ hybridization.

Results. In both models of bFGF upregulation, the ERG b-wave was suppressed over a wide stimulus range and in light- and dark-adapted conditions. The a-wave was not suppressed by either procedure and at the brightest intensities was enhanced by both procedures. In nerve-sectioned eyes, outer retina appeared normal histologically, but levels of bFGF protein in the inner and outer nuclear layers were raised, whereas bFGF mRNA levels remained unchanged. In both models, levels of synaptophysin in the outer plexiform layer and of cytochrome oxidase in inner segments were raised in association with increases in bFGF protein levels.

Conclusions. bFGF increased the ability of photoreceptors to respond to light but attenuated the transmission of this response to inner retinal cells, presumably by blocking the photoreceptor–bipolar synapse. If the expression of bFGF protein is upregulated in human photoreceptor dystrophies, it may contribute a reversible component to the loss of vision. The relationship between these actions of bFGF and its ability to protect photoreceptors from stress remains to be established. (*Invest Ophthalmol Vis Sci.* 1999;40:2088–2099)

Evidence that the retina reacts to the stresses of its normal function by upregulating protective mechanisms came from the studies of Penn and Anderson,[1] who showed that moderate levels of light experienced in a normal daily cycle both damage the genetically normal retina (reducing the photoreceptor population) and increase its resistance to further damage. Such evidence indicates a process of basic biologic interest and makes self-protection an important issue in understanding the causes of photoreceptor dystrophies.

Early evidence that bFGF is protective to photoreceptors came from the demonstration that bFGF applied exogenously to photoreceptors of the rat eye promotes their survival in the face of genetic mutation[2] and light damage.[3] Evidence that photoreceptors protect themselves from damage by expressing bFGF endogenously can be traced to the observation of Bush and Williams[4] that optic nerve section makes the photoreceptors of the affected eye resistant to light damage. Kostyk et al.[5] related this protective effect of nerve section to bFGF, showing that nerve section induces an increase in bFGF protein in the outer nuclear layer (ONL). Subsequent studies have shown with increasing specificity that stress upregulates the expression of bFGF protein in the retina, and that this upregulation is protective. Small laser lesions that slow the degeneration of surrounding photoreceptors in the retina of the Royal College of Surgeons rat[6] also upregulate bFGF protein levels in the rescued cells.[7,8] Photoreceptors upregulate their expression of bFGF protein during genetic and light-induced dystrophy of the mouse retina.[9,10] The retina upregulates its expression of bFGF protein in response to light damage, and that upregulation is protective.[11] bFGF is among the factors upregulated by the retina in response to mechanical damage, and their upregulation is also protective.[12]

bFGF may also contribute to the protective effect of bright but physiological levels of daily light experience.[1] Recent evidence[13] suggests that photoreceptors react to normal levels of daily light by expressing bFGF protein in amounts directly related to the intensity of the daily light. Further, this expression of bFGF in photoreceptors appears to determine the severity of damage caused to them when they are stressed by prolonged bright light. The mechanisms by which factors such as bFGF protect photoreceptors are, however, still unknown. The present experiments were designed to assess the impact of bFGF on the structure and function of photoreceptors, as a step toward identifying the mechanisms of protection by bFGF.

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METHODS

All procedures conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Optic Nerve Section

Nerve section was performed on 11 adult rats under surgical anesthesia (tribromoethanol, 1.5 mg/100g, by intraperitoneal injection as a 2% solution in buffered saline). The optic nerve was sectioned in the orbit several millimeters behind the eyeball. To ensure that the section spared the vascular supply to the eye, the following steps were taken: The nerve was approached from its superior aspect, through an incision in the upper eyelid. It was visualized in the orbit by blunt dissection. The nerve was raised dorsally and laterally to separate it from the short ciliary vessels, which approach from its medioventral aspect. The nerve was cut behind the entry of the short ciliary arteries into the nerve. Subsequent histology confirmed that the inner retina and its vessels (which degenerate if the retinal circulation is blocked[14]) remained intact (described later).

Eyeball Injection

The eyeball was injected in 16 adult rats and 2 adult cats, under surgical anesthesia (as for nerve section). In each case bFGF (human recombinant, 1 μg/μl; Sigma, St. Louis, MO) in PBS was injected into the vitreous humor of the right eye, using a fine needle and following protocols of earlier studies.[23] In some animals the left eye was injected with the same volume of PBS.

Electroretinography

Recordings were made 3 to 4 weeks after optic nerve section and 1 to 10 days after intravitreal injection. Rats were anesthetized with urethane (1200 mg/kg), kept warm by a feedback-controlled electric blanket and placed in a conventional head holder. In cats, anesthesia was induced by an intramuscular injection of ketamine (30 mg/kg; Ketalar, Parke–Davis, Morris Plains, NJ). An endotracheal tube and a venous cannula were then inserted. The animal was paralyzed with an intravenous infusion of a 0.2% solution of pancuronium bromide (Pavulon; Organon Teknika, Durham, NC) at a rate of 0.1 to 0.2 ml/kg per hour and artificially ventilated. Anesthesia was maintained throughout the recording session by intravenous infusion of sodium thiopental (3.5 mg/kg per hour). Silver wire electrodes were introduced into the vitreous humor through a minimal incision on the lateral side of the eyeball. Recordings were made from left and right eyes separately, with the other eye fully covered. Recordings were made between the two vitreous electrodes or between the electrode in the vitreous and an electrode placed on the tongue. The band-pass of the amplifier was 0.1 Hz to 500 Hz.

Rats were dark-adapted for 40 minutes before recording commenced, and darkness was maintained throughout except for the light stimulus. Three modes of light stimulation were used. In one, dark-adapted responses were evoked with an 8-msec flash of a monitor screen subtending approximately 30° at the eye and of intensity up to 28 cd/m^2. Interstimulus intervals were 2 minutes or longer, and during this time the screen was dark. In the second mode, light-adapted responses were obtained using the same screen stimulator. The stimulus was a square-wave variation of intensity of mean brightness 18 cd/m^2 (maximum range, 0–36 cd/m^2. The frequency of stimulation was 0.5 Hz, and the screen brightness remained high for 1 second, then low for 1 second. In the third series of experiments dark-adapted responses were obtained using a flash unit that produced flashes of 1.5-msec duration and 15,000 cd/m^2 intensity, equivalent to 105,000 scotopic troland/sec. Neutral density filters were used to attenuate the flash for intensity–response relationships. The background was kept dark, and the interstimulus interval was at least 2 minutes. The a-wave was measured from the baseline to the first negative peak, and the b-wave was measured from negative to positive peaks. In a few experiments we recorded ERG responses to bright flashes with an amplifier bandwidth extended up to 3 kHz. In these instances oscillatory potentials could be observed in the normal ERG, but they were almost absent in the optic nerve sectioned eye.

Histology and Immunohistochemistry

Retinas were fixed by immersion of the globe in 4% paraformaldehyde in PBS at pH 7.4 for 1 to 6 hours. Some eyes were then embedded in celloidin, sectioned at 30 μm, and stained with cresyl violet. Other eyes were placed in 20% sucrose until they sank, frozen embedded in TissueTek (Miles, Elkhart, IN), and cryosectioned at 20 μm for use in immunocytochemistry. Retinas were labeled for bFGF in steps, according to protocols published previously.[15] Sections were labeled with antibodies to cytochrome oxidase (CO; Molecular Probes, Eugene, OR) at 1 μg/ml, to rod opsin (Rho4D2, gift from Robert S. Molday, University of British Columbia, Vancouver, Canada) at 1:100, or to synaptophysin (Dako, Carpinteria, CA) at 1:100. Some sections were second labeled with a DNA- specific dye (SYTO-12 or TOTO-3, diluted 1:1000 and applied for 20 seconds; Molecular Probes) or a biotinylated peanut agglutinin (PNA) lectin (Vector Laboratories, Burlingame, CA). PNA labels cone sheaths specifically.[16] It was applied at a final dilution of 400 μg/ml in PBS, followed by streptavidin Cy2 or Cy3.

In Situ Hybridization

cRNA probes were prepared from a 477-bp cDNA strand corresponding to nucleotides 533 to 1009 of a rat ovarian bFGF cDNA. This cDNA incorporates the complete bFGF coding sequence and a 75 nucleotide 3′ flanking sequence. The strand was cloned into a vector (pBluescript SK$^+$; Stratagene; La Jolla, CA). The detailed procedures used have been published.[15]

Quantitation of Signals

Molecule-specific signals from immunolabeled proteins were quantified using NIH Image software (the Analysis tool; Bethesda, MD) applied to digitized images obtained by confocal microscopy. The quantitative comparisons in Figures 7, 8, and 9 were made in the following conditions. The sections from the two eyes to be compared were fixed and labeled by identical protocols in the same run and imaged by the confocal microscope with the photomultiplier tube settings constant. Any subsequent optimization of the image was identical in the two images, and identical analytical tools were used on the digitized images. A similar approach was taken to the quantification of probes hybridized to bFGF mRNA, except that the label used was not fluorescent, but the nitroblue tetrazolium chloride chromogen, and the images were digitized on a conventional microscope.

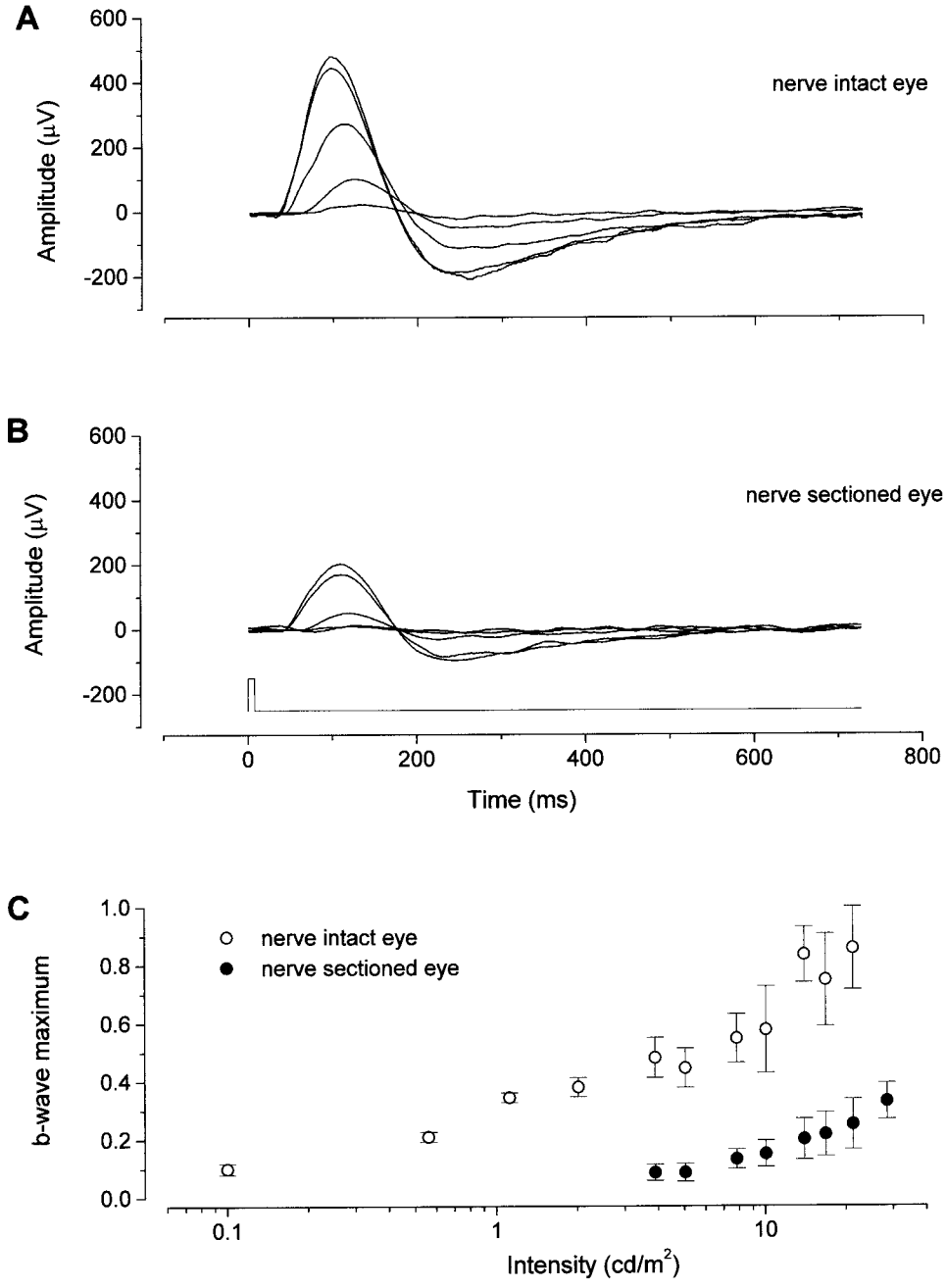


Figure 1. Response of nerve-sectioned and nerve-intact eyes to dim flashes (rat, dark-adapted conditions). The flashes were of 8 msec duration. (**A**) Responses of the nerve-intact eye to flashes of intensity 0.11, 0.56, 5, 14, and 28 cd/m^2. Each trace is the average of five successive responses. (**B**) Responses of the nerve-sectioned eye to flashes of the same intensities as in (**A**). (**C**) Normalized b-wave amplitudes averaged over four experiments, for nerve-intact and nerve-sectioned eyes. Error bars, SE.

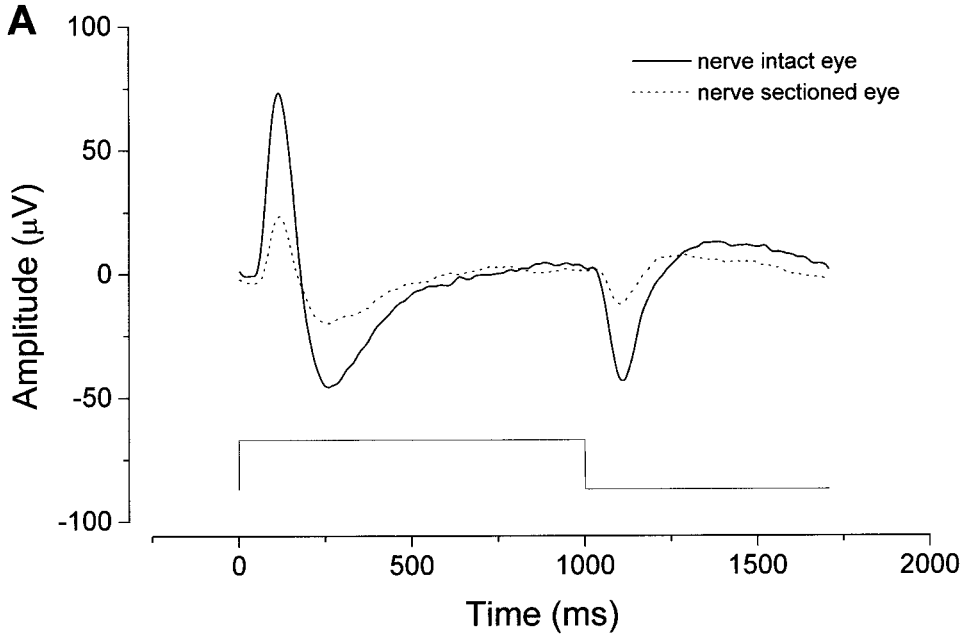
Results

Effect of Nerve Section on the ERG Evoked by Dim Stimuli

Dark-Adapted Responses. Figure 1 shows dark-adapted responses to an 8-msec flash of intensity 0.11 to 28 cd/m^2 recorded from the left (Fig. 1**A**) and right (Fig. 1**B**) eyes of a rat in which the right nerve had been sectioned 3 to 4 weeks previously. Responses from the two eyes are shown to the same five intensities of the flash. The general shape of the response did not vary markedly between eyes over the intensity range used, but response amplitude was consistently smaller in the nerve-sectioned eye. In each of four experiments, responses were averaged over four stimulus cycles; Figure 1C shows normalized mean amplitudes and SEMs. Extrapolation of the data points in Figure 1C to the abscissa suggests a difference in threshold of approximately 1.2 log units between nerve-intact and nerve-sectioned eyes. Over the stimulus intensity range used, the data series from the two eyes are also separated along the abscissa by approximately 1.2 log units.

Light-Adapted Responses. Figure 2A shows light-adapted responses evoked in a nerve-sectioned rat by a square-wave modulation (0.5 Hz; mean luminance, 18 cd/m^2; amplitude, 0–36 cd/m^2) stimulus. The traces shown are averages of 10 successive responses from right (nerve-sectioned) and left (intact) eyes in one experiment. Again, nerve section did not markedly change the shape of the response but reduced the amplitude of both OFF and ON components.

The effect of sham operations, in which the optic nerve was left intact, was assessed using light-adapted responses (Fig.



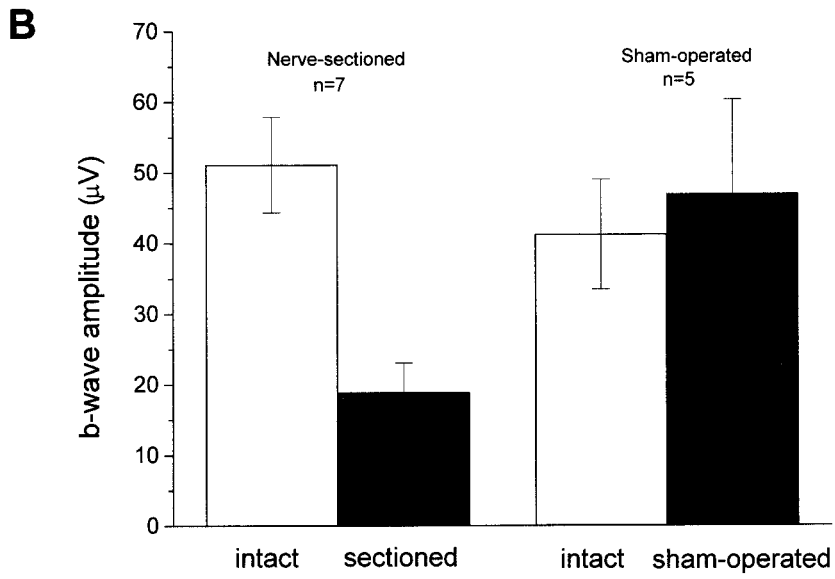


FIGURE 2. (**A**) Response of nerve-sectioned and nerve-intact eyes to dim flashes (rat, light-adapted conditions). The stimulus was the square modulation of the brightness of a screen. Mean brightness was maintained at 18 cd/m^2; modulation was 0 to 36 cd/m^2. (**B**) Summary data for light-adapted experiments on nerve-sectioned and sham-treated rats. Error bar, SEM. Averaged over seven experiments using the same stimulus parameters as in (**A**), the b-wave was larger in the nerve-intact eye than in the nerve-sectioned eye. Averaged over five sham operation experiments, there was no difference in b-wave amplitude between control and surgical eyes.

2B). Measured using the ON-response evoked by a 0 to 36 cd/m^2 square wave, the response of the nerve-sectioned eye averaged over seven animals was significantly smaller than that of the nerve-intact eye (Fig. 2B, left-hand pair of bars). By contrast, the response of the sham procedure eye averaged over five animals was not significantly reduced (Fig. 2B, right-hand pair of bars).

Effect of Nerve Section on the ERG Evoked by Bright Flashes

Figures 3A and 3B show dark-adapted responses from the nerve-intact (A) and nerve-sectioned (B) eyes of a rat to 1.5-msec flashes of intensities 3.5, 7, and 28 cd/m^2. As in Figure 1, responses to these dim flashes were dominated by the b-wave and were of lower amplitude in the nerve-sectioned eye. When flashes of intensity 112 to 7500 cd/m^2 were used, the a-wave of the response became identifiable and then prominent (Figs. 3C, 3D). Over the range of intensities for which it could be identified (>100 cd/m^2) the a-wave was detected at lower intensities in the nerve-sectioned eye, was never less in the nerve-sectioned eye, and at the highest intensities (7500 cd/m^2) was clearly larger in the nerve-sectioned eye. These features are all apparent when comparing Figures 3C and 3D.

This apparent enhancement of the a-wave was evident in all five animals tested to these high intensities and is summarized in Figure 6. The b-wave evoked by stimuli brighter than 112 cd/m^2 appeared to be slightly reduced in amplitude in the

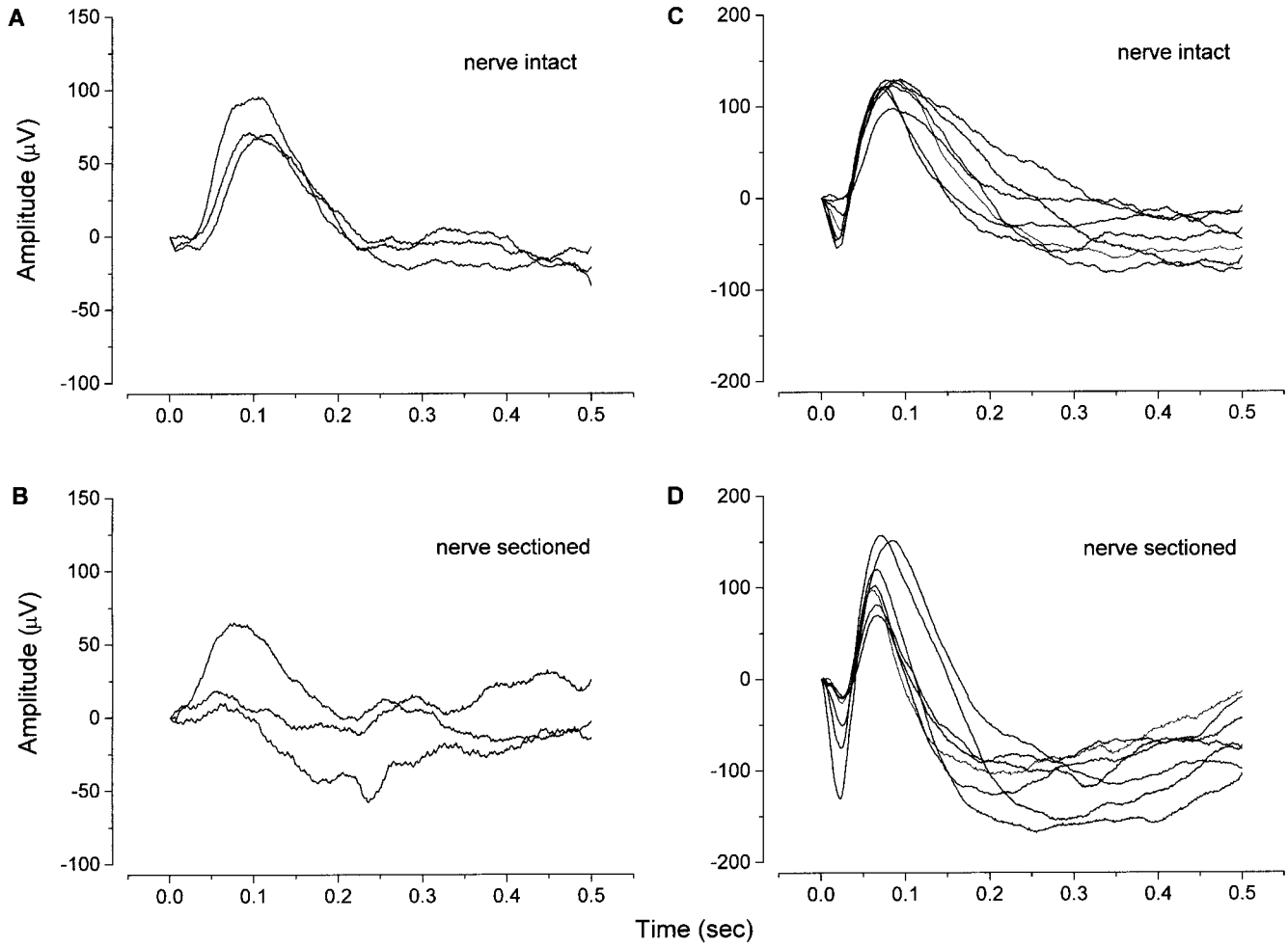


FIGURE 3. Intensity–response series for a nerve-sectioned rat, using a brief (1.5-msec) flash. Each trace shown is the average of five successive traces. (**A**, **B**) Responses from the nerve-intact (**A**) and nerve-sectioned (**B**) eyes to three low-intensity flashes (3.5, 7, and 28 cd/m^2). (**C**, **D**) Responses from the nerve-intact (**C**) and nerve-sectioned (**D**) eyes to seven high-intensity flashes (112, 203, 406, 812, 1625, 3250, and 7500 cd/m^2).

nerve-sectioned eye in some animals and slightly enhanced in others. Given that a- and b-wave overlap temporally, a conservative conclusion is that the b-wave evoked by bright stimuli is not consistently affected in amplitude by nerve section. However, given the evidence just discussed that the a-wave is enhanced by sectioning, and the reasonable assumption that the b-wave is generated by retinal cells activated by the a-wave, the present results suggest that the b-wave evoked by these high-intensity stimuli is suppressed by nerve section.

Effect of bFGF Injection on the ERG Evoked by Dim Stimuli

In rats studied 1 and 2 days after injection, no suppression of the ERG was apparent (data not shown). In animals studied 3 to 10 days after injection, the ERG was reduced in amplitude in 13 of 16 experiments. Qualitatively, the responses evoked by a dim flash (28 cd/m^2) show a suppression of b-wave similar to that seen after nerve section (Fig. 1). The response was dominated by the b-wave, and bFGF injection reduced its amplitude without altering its overall shape. The responses shown in Figure 4 were recorded in dark-adapted conditions. Similar suppression was seen in the light-adapted conditions described for the nerve-section experiments (Fig. 2A). Individual traces are not shown, but light- and dark-adapted results are summarized in Figure 6C.

Two experiments were performed in cats because the larger size of the eye makes the delivery of bFGF into the vitreous humor much easier, with less chance of bleeding or injury to structures such as the lens. In both cats clean delivery of the bFGF was attained, and in both, the ERGs evoked by stimuli by the square wave (0.5Hz; 0–36 cd/m^2) were markedly suppressed, to less than 50% of control values. Individual traces are not shown, but the data are included in Figure 6C.

Effect of bFGF Injection on the ERG Evoked by Bright Flashes

ERGs elicited by 1.5-msec flashes of 0.3 to 28 cd/m^2 are shown in Figures 5A, and 5B for vehicle- and bFGF-injected eyes. At these low intensities the ERG was dominated by the b-wave and, as in Figure 4, was markedly suppressed by bFGF injection. When flashes of intensity 112 to 7500 cd/m^2 were used, the a-wave of the response became identifiable and at higher intensities became prominent (Fig. 5C, 5D). Over the range of intensities for which it could be identified ($>$100 cd/m^2) the a-wave appeared at lower intensities in the bFGF-injected eye, was never less in the bFGF-injected eye, and at the highest

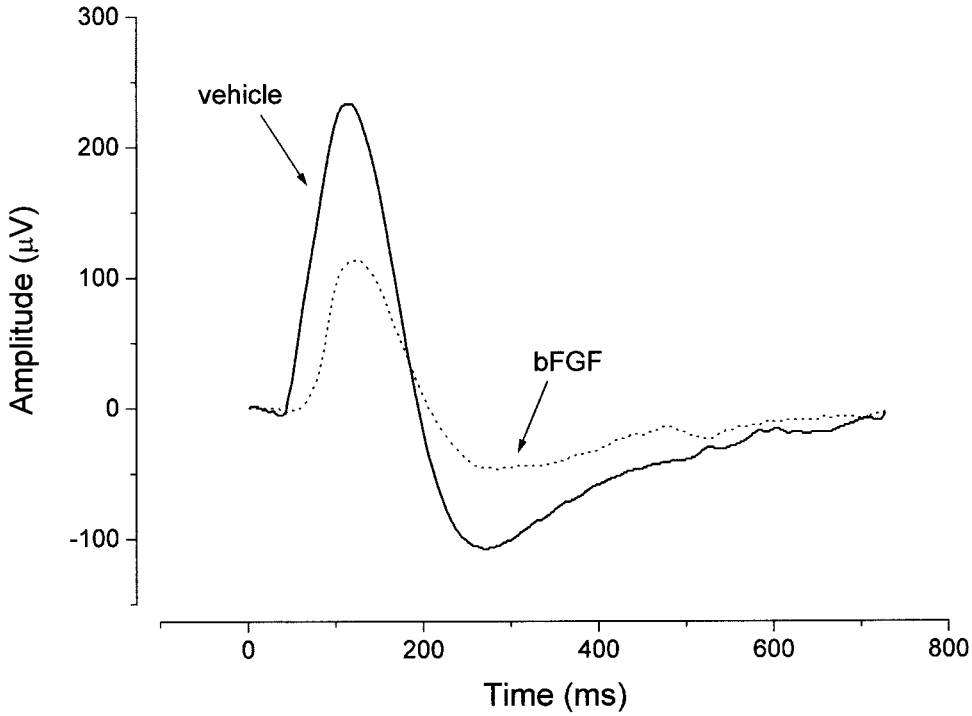


FIGURE 4. Responses of vehicle- and bFGF-injected eyes to a flash of 8 msec duration and 28 cd/m^2 intensity. Traces show the average of five successive responses.

intensities (7500 cd/m^2) was clearly larger in the bFGF-injected eye. The b-wave evoked by stimuli brighter than 100 cd/m^2 appeared to be slightly reduced in amplitude in the bFGF-injected eye in some animals and slightly enhanced in others. Again, when the larger size of the a-wave evoked by bright stimuli is taken into account, these results suggest that the b-wave elicited by very bright stimuli is suppressed to some degree by bFGF injection.

Summary and Controls

Nerve-Sectioned Data. Figure 6A summarizes the suppression of the b-wave to low-intensity flashes. Plotting b-wave amplitude from the sectioned eye against the nerve-intact eye for both light- and dark-adapted responses, the data points all fall below the oblique left-equals-right line, for both light- and dark-adapted responses. Using the same convention (Fig. 6B) for five experiments with bright flashes (duration, 1.5 msec; intensity, 7500 cd/m^2), the a-wave was larger in the nerve-sectioned eye in all five experiments, whereas the b-wave was not consistently suppressed or enhanced.

Eye-Injection Data. Figure 6C summarizes present results for low-intensity stimuli. The effect of bFGF injection was more variable than that of nerve section, but a reduction of amplitude was apparent in the bFGF-injected eye in 13 of the 16 rats studied 3 to 10 days after injection and in both cat experiments. The reduction was apparent in both light-adapted (open symbol) and dark-adapted (closed symbol) conditions. In some cases the reduction was as marked as that after nerve section. Injections of vehicle were made into the left eye (as well as of bFGF into the right eye) of 4 of the 18 rats studied and in both the cat experiments. In all six cases the b-wave amplitude of the bFGF-injected eye was less than 50% of its value in the vehicle-injected eye. Using the same convention (Fig. 6D) for four experiments with bright flashes (duration, 1.5 msec; intensity 7500 cd/m^2), the a-wave was larger in the bFGF-injected eye in all four experiments, whereas the b-wave was not consistently suppressed or enhanced.

Effect of Nerve Section on Retinal Structure

With a Nissl stain (Figs. 7A, 7B) the middle and outer layers of the retina appeared unaffected by nerve section. At higher power, a DNA label (Figs. 7C, 7D) showed large somas in the ganglion cell layer (g) of the nerve-intact retina but was absent from the nerve-sectioned retina, suggesting the retrograde degeneration of ganglion cells. In Figures 7E and 7F a DNA dye (blue) confirmed that the ONL was normal in general morphology after nerve section. The labeling of outer segments by the anti-opsin antibody (green) suggests no abnormality in the outer segments. The labeling of the inner segments and the outer plexiform layer (OPL) by the antibody to CO (red) appeared normal in location in the nerve-sectioned eye (compare Figs. 7E and 7F). The CO labeling appeared enhanced in intensity in the nerve-sectioned retina but showed no evidence of morphologic abnormality.

Effects of Nerve Section on bFGF Expression

bFGF Protein. Figures 8A and 8B show the result obtained in three of the six animals examined. In the nerve-intact retina (Fig. 8B), bFGF distribution resembled that seen in the normal rat retina.[13] bFGF was prominent in somas in the inner nuclear layer (INL) shown by previous workers[7] to be the somas of Müller cells. bFGF was evident also in the inner segments of photoreceptors but not in their somas in the ONL. In the nerve-sectioned retina (Fig. 8A), bFGF appeared more strongly expressed in Müller cell somas, in inner segments, and in the ONL. These trends, shown quantitatively in Figure 8C, suggest that the expression of bFGF protein was upregulated by nerve section in Müller cell somas, in the ONL, and in the inner segments.

In the other three animals, bFGF protein levels in the nerve-sectioned retina appeared to be elevated in the ONL, as

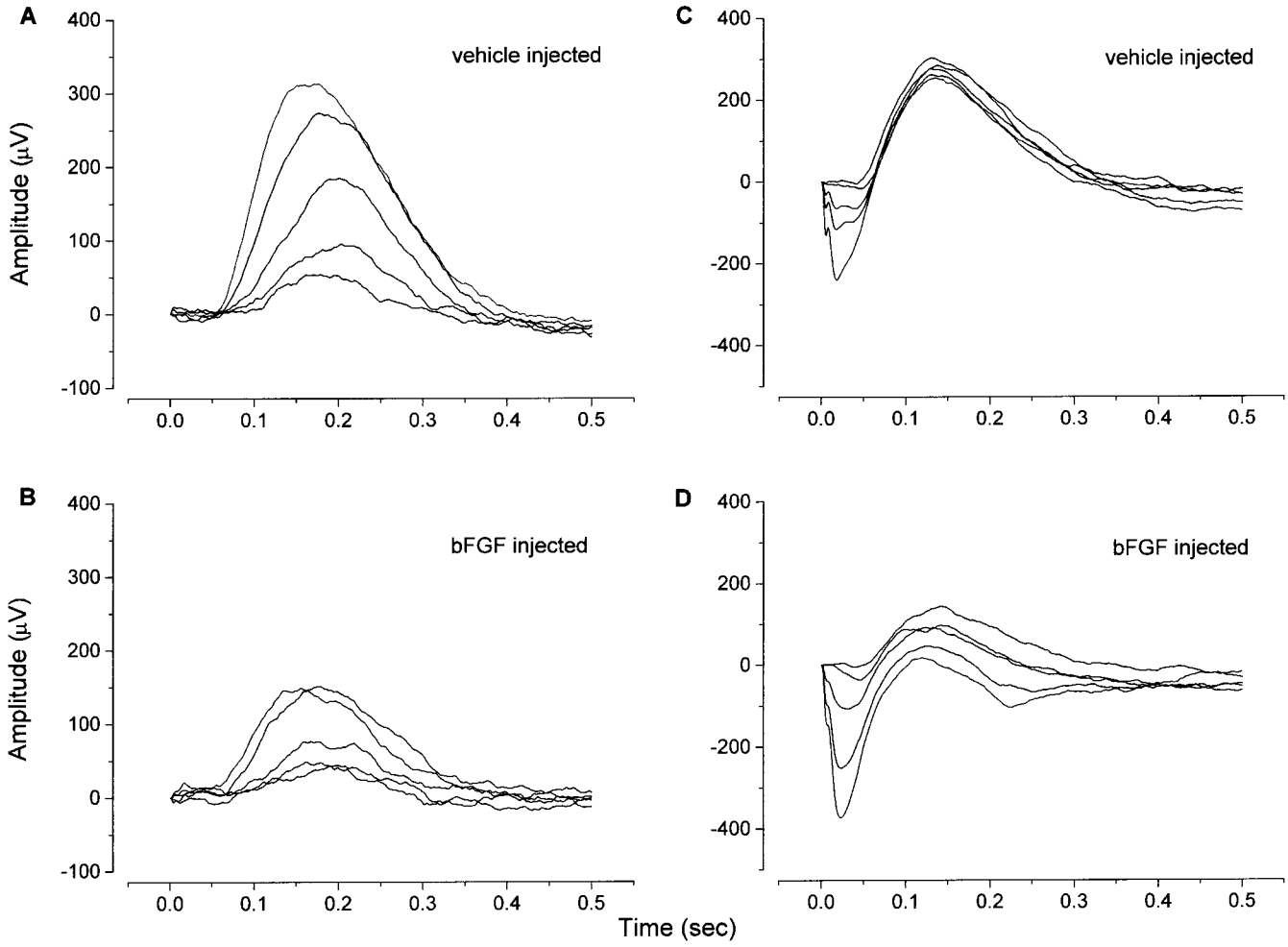


FIGURE 5. Intensity–response series for a bFGF-injected rat, using a brief (1.5-msec) flash. Each trace shown is the average of five successive traces. (**A**, **B**) Responses from the vehicle-injected (**A**) and bFGF-injected (**B**) eyes to five low-intensity flashes (0.3, 0.8, 3.5, 14, and 28 cd/m^2). (**C**, **D**) Responses from the vehicle-injected (**C**) and bFGF-injected (**D**) eyes to five high-intensity flashes (112, 406, 1625, 3250, and 7500 cd/m^2).

in Figure 8A. However, bFGF protein levels in the nerve-intact eye appeared abnormal. The concentration of bFGF in Müller cell somas had dispersed along Müller cell processes, so that their inner feet and the INL appear strongly labeled (not illustrated).

bFGF mRNA. Comparison of bFGF mRNA levels in nerve-intact and nerve-sectioned retinas is shown in Figures 8D, 8E, and 8F. In neither of two animals examined was there evidence that the expression of bFGF mRNA was upregulated in the nerve-sectioned retina. In particular, the level of bFGF mRNA in the somas or inner segments of photoreceptors was no higher in the nerve-sectioned retina, despite the concentration there of bFGF protein.

Upregulation of bFGF Protein Expression Cones and Rods. Granules labeled (green) for bFGF protein appeared to be present in every soma of the ONL (o in Figs. 8G, 8H, 8I; left panels). Sheaths of cone inner and outer segments, labeled (Figs. 8G, 8H, 8I; red) with the PNA lectin, could occasionally be traced to their somas (8G, 8H; arrows in right panels). These somas also contained bFGF granules in their cytoplasm (8G, 8H; lower right panels). The outer segments of both rods (Fig. 8I; small arrows) and cones (larger arrow) were also bFGF$^+$.

Effects of Nerve Section and bFGF Injection on Activity-Related Molecules

Nerve section induced upregulation of the expression of both CO and synaptophysin. Comparing Figures 9A and 9B, CO levels in the inner segments and OPL were higher in the nerve-sectioned than in the nerve-intact retina. Figure 9C shows this comparison quantitatively. Nerve section also induced an upregulation of the expression of synaptophysin in the OPL (Figs. 9D, 9E, 9F). Both results were consistent in the four animals examined. bFGF injection mimicked optic nerve section in upregulating CO expression in the OPL and inner segments (Fig. 9G) and synaptophysin expression in the OPL (Fig. 9H). This result was consistent in the three bFGF-injected animals examined.

DISCUSSION

The two models of upregulation of bFGF protein levels studied were chosen because, in both, photoreceptors are protected from damage.[2–4] We hoped that identifying the impact of bFGF on photoreceptors in such models might help identify actions

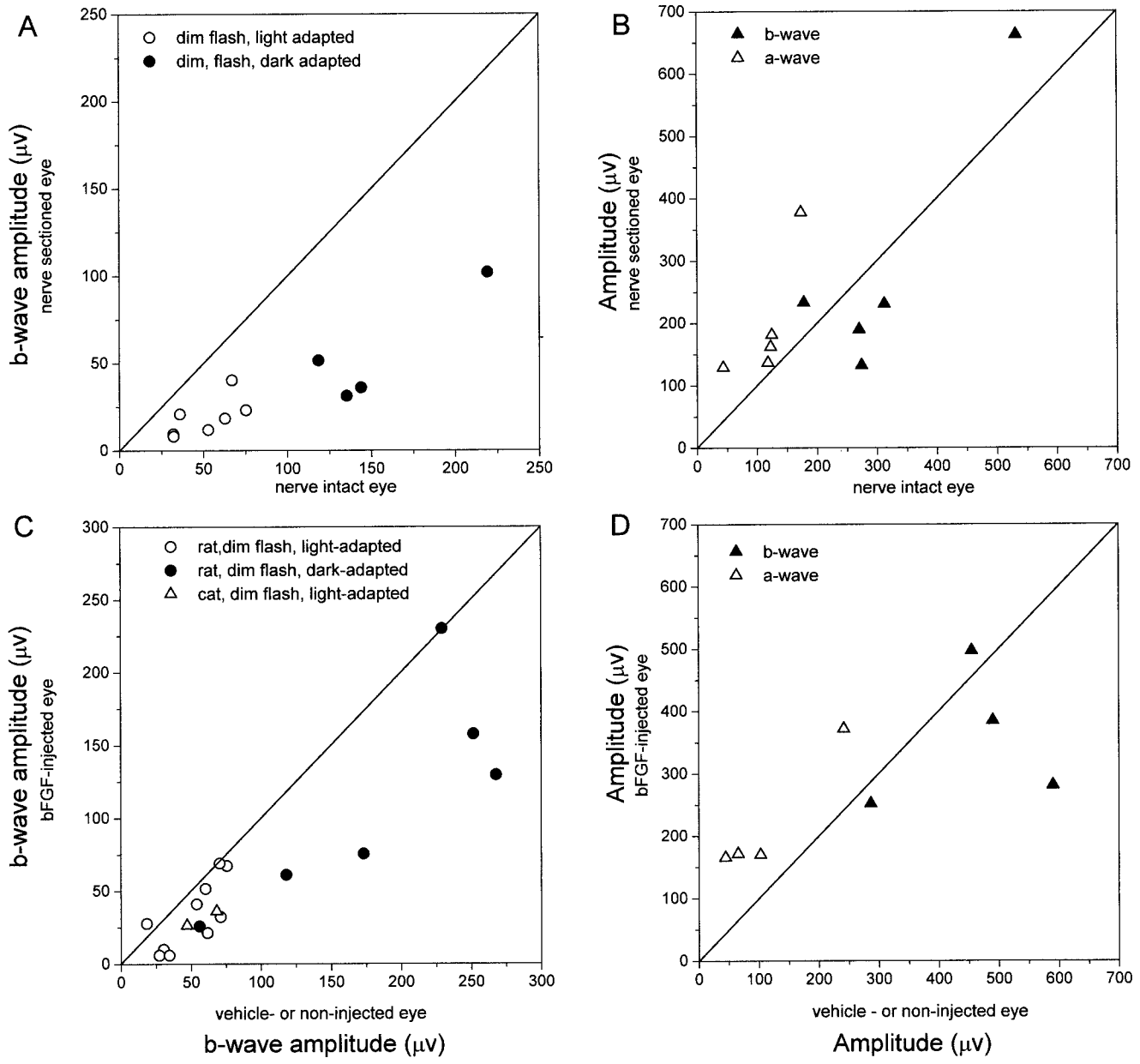


Figure 6. Summary plots for the present ERG experiments. (**A**) Nerve-sectioned experiments, using a dim flash (duration, 8 msec; intensity, 28 cd/m^2) in dark-adapted conditions or a square wave (0.5 Hz; 0–36 cd/m^2) modulation of the stimulus screen (light-adapted conditions). Each data point plots the amplitude of the b-wave from the nerve-sectioned eye against the amplitude of the b-wave from the nerve-intact eye. (**B**) Nerve-sectioned experiments, using a bright (7500 cd/m^2) flash. Each point plots the amplitude of the response (a- or b-wave, absolute values) obtained from the nerve-sectioned eye against the amplitude of the same wave in the nerve-intact eye. (**C**) bFGF injection experiments using a dim flash (duration, 8 msec; intensity, 28 cd/m^2) in dark-adapted conditions, or a square wave (0.5 Hz; 0–36 cd/m^2) modulation of the stimulus screen (light-adapted conditions). Each data point plots the amplitude of the b-wave from the bFGF-injected eye against the amplitude of the b-wave in the other (vehicle-injected or noninjected) eye. (**D**) bFGF injection experiments, using a bright (7500 cd/m^2) flash. Each point plots the amplitude of the response (a- or b-wave, absolute values) obtained from the bFGF-injected eye against the amplitude of the same wave in the other (vehicle-injected or noninjected) eye.

of bFGF that mediate protection. The nerve-section model is attractive because the upregulation of the expression of bFGF protein is endogenous, but the retinal expression of other factors may also be changed, so that the effects observed may not be specific to bFGF. The eye-injection model is more specific to bFGF but involves structural damage to the eye. Taken together, present results suggest that bFGF induces specific functional changes in the retina, suppressing the b-wave while maintaining or enhancing the a-wave and upregulating the expression of proteins (CO, synaptophysin) closely related to the signaling function of photoreceptors. The contrast between the actions of bFGF in suppressing the b-wave while increasing the photoreceptors' signaling capacity is striking, but the significance of the contrast and the link between these several effects and the protective action of bFGF remain enigmatic.

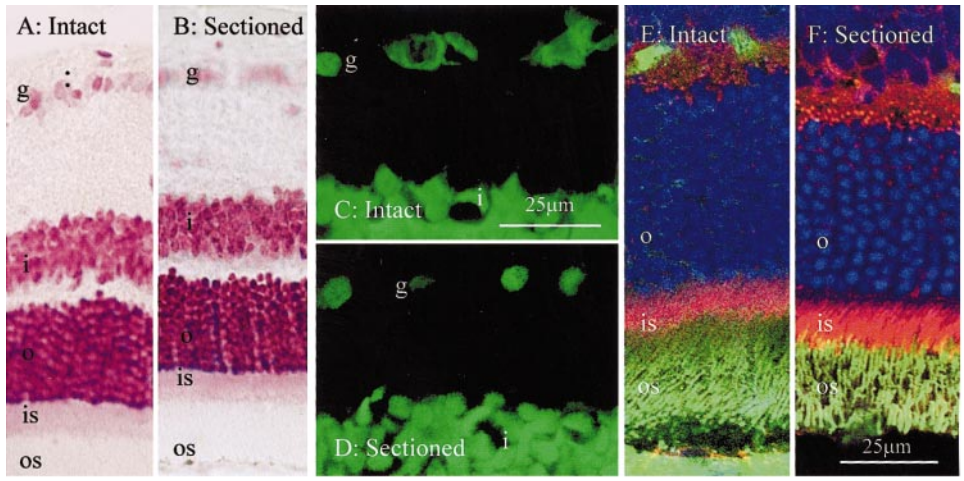


FIGURE 7. Effect of nerve-section on structure of outer retina (**A**, **B**) Nerve section did not disturb the general histology of the outer retina. Stain is cresyl violet in celloidin sections. Ganglion cells are present in (**B**) but are out of focus. (**C**, **D**) Nerve section reduced the size of somas in the ganglion cell layer (g), presumably by causing the retrograde degeneration of ganglion cells. Label is SYTO-12 (Molecular Probes, Eugene, OR). (**E**, **F**) Nerve section did not reduce the length or opsin labeling (*green*) of outer segments, and increased CO labeling (*red*) in the inner segments. The *green*-labeled structures in the OPL in (**E**) are blood vessels, which often label, presumably nonspecifically, with this antibody. is, inner segments, o, outer nuclear layer; i, inner nuclear layer; g, ganglion cell layer.

Present results confirm those in a previous report[5] of upregulation of bFGF protein expression in the ONL of rat retina after section of the optic nerve. There appear, however, to be no previous reports of the effect of nerve section on the ERG of the rat. Several previous studies in the cat[17,18] and rabbit[19] reported no effect of nerve section on the major components of the ERG. One study[20] reported a transient suppression of the rabbit ERG after nerve section. The present evidence of major effects of nerve section on the rat ERG may reflect a species difference or our choice to test the retina at the particular time (3–4 weeks after nerve damage) at which the protection of photoreceptors is optimal[4] and the upregulation of bFGF protein expression in the ONL is prominent.[5]

Regulation of CO and Synaptophysin

The upregulation of the expression of CO and synaptophysin proteins induced by nerve section and bFGF injection was unexpected but consistent. The increase in CO protein was apparent in inner segments, where the enzyme plays a major role in the high oxidative metabolism of the inner segment mitochondria[21] required to maintain the function of the outer segment.[22,23] The upregulation of synaptophysin expression was prominent in the OPL, where the protein forms part of the membrane of presynaptic vesicles[24] in the terminals of photoreceptor axons. The upregulation of CO expression in the inner segments suggests that the cell is able to generate adenosine triphosphate by oxidative metabolism in larger quantities and may be supporting high levels of dark current through the outer segments. If so, the cell should be able to generate a stronger than normal a-wave. The upregulation of synaptophysin expression in the OPL suggests that synaptic vesicles are more numerous than usual in the axon terminals of the photoreceptors. Their accumulation could result from an increase in vesicle formation or from a slowing of their release.

Suppression of the b-Wave: Site and Mechanism

The most likely site at which bFGF could selectively suppress the b-wave would seem to be the OPL, but present results provide only limited evidence of the mechanisms involved. bFGF may reduce the light-induced modulation of the photoreceptors' release of glutamate, consistent with the bFGF-induced accumulation noted earlier of synaptophysin-labeled structures, presumably vesicles, in the OPL. Alternatively, bFGF may block the binding of glutamate to its receptors on bipolar cells. Present evidence that both ON and OFF components of the ERG were reduced by bFGF (Fig. 2A) indicates that signals generated through both metabotropic (ON) and ionotropic (OFF) glutamate receptors in bipolar cells (reviewed in Ref. 25) are both attenuated by increases in bFGF protein levels. Further analysis of the action of bFGF requires additional study, however. Recent evidence[26] that the growth factor brain-derived neurotrophic factor (BDNF) can upregulate the expression of Ca^{2+} channels in motoneurons raises the possibility that bFGF may regulate channel densities in retinal neurons, but this possibility also requires further study.

Suppression of the b-Wave: Implications

The suppression of the b-wave induced by bFGF was clearest in response to stimuli with the low contrast levels common in normal vision. It seems possible therefore that increases in the levels of bFGF protein in the retina degrade normal vision. If bFGF levels are raised in the dystrophic human retina, as in the dystrophic rat and mouse,[9,10] then one component of the blindness of retinitis pigmentosa may be a reversible bFGF-induced suppression of the ability of surviving photoreceptors to activate the inner layers of the retina. Human retina expresses bFGF,[27] although there is as yet no evidence of (or against) upregulation of bFGF expression in human retinitis pigmentosa. Conversely if, as we have argued elsewhere,[13]

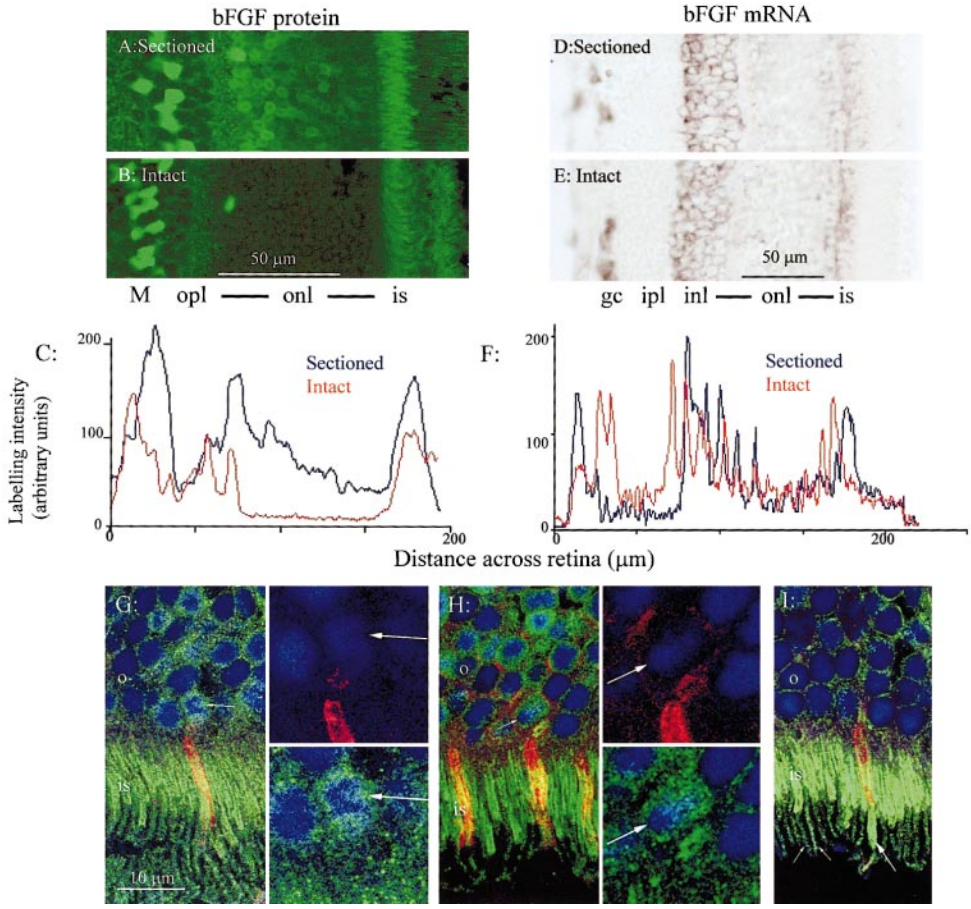


FIGURE 8. Effect of nerve section on bFGF expression in the retina. (**A**, **B**) Representative cross sections of nerve-sectioned and nerve-intact retinas, immunolabeled for bFGF protein. (**C**) Labeling intensity measured along lines 9 pixels wide oriented across the layers in (**A**) and (**B**). The graph has been set in size so that the abscissa matches in extent the sections in (**A**) and (**B**). (**D**, **E**) Representative cross sections of nerve-sectioned and nerve-intact retinas, hybridized with a cRNA probe to bFGF mRNA. (**F**) Labeling intensity measured along lines 9 pixels wide oriented across the retinal layers. The graph has been set in size so that the abscissa matches in extent the sections in (**D**) and (**E**). (**G**) Evidence that bFGF protein expression was upregulated in cones as well as rods. The *left* panel shows a cone sheath (*red*, PNA labeling) stretching across the layer of inner segments (is, strongly labeled *green* for bFGF) leading to a soma of which the nucleus (labeled *blue* with the DNA dye TOTO-3) is shown at higher magnification in the two *right* panels (*arrows*). In the *upper right* panel the *green* bFGF immunolabel is suppressed. In the lower *right* panel the *red* PNA label is suppressed. The cone nucleus is surrounded by bFGF granules (*green*) as richly as other (rod) nuclei in the ONL (o). (**H**) A second example of bFGF protein levels in a cone soma, shown with the same conventions as in (**G**). (**I**) Evidence of bFGF immunolabeling (*green*) in the outer segment of a cone (*arrow*) identified as a cone by its association with a PNA-labeled (*red*) cone sheath. Nearby rod outer segments also show labeling for bFGF. M, Müller cells; gc, ganglion cell layer; inl, inner nuclear layer; onl, outer nuclear layer; is, inner segments.

photoreceptors surviving in dystrophic retinas are under constant stress and stress upregulates bFGF protein expression (reviewed in the introduction), then measures that relieve that stress may not only slow the dystrophy but may, by reducing bFGF levels, lead to a limited improvement in visual performance.

Regulation of bFGF Protein Levels

Comparison of the bFGF protein levels in the retina (Figs. 7A, 7B) with bFGF mRNA levels (Figs. 7C, 7D) was surprising in two ways. First, bFGF protein concentrates markedly in Müller somas of the INL, whereas the mRNA is more diffusely distributed in apparently all cells of the layer. This seems to suggest that the high level of bFGF protein in Müller cells is the result of a posttranslational movement of bFGF into Müller cells. The mechanism of such movement is not known, and its function can only be guessed. One possibility is that Müller cells store bFGF until the retina is stressed or damaged, and then distribute it by way of their extensive processes.

Second, we consistently observed major differences between nerve-sectioned and nerve-intact retinas in the levels of bFGF protein in the ONL and inner segments (Figs. 8A, 8B, 8C) and little difference, if any, in mRNA levels (Figs. 8D, 8E, 8F). This contrast suggests that these differences in protein levels also result from posttranslational mechanisms,

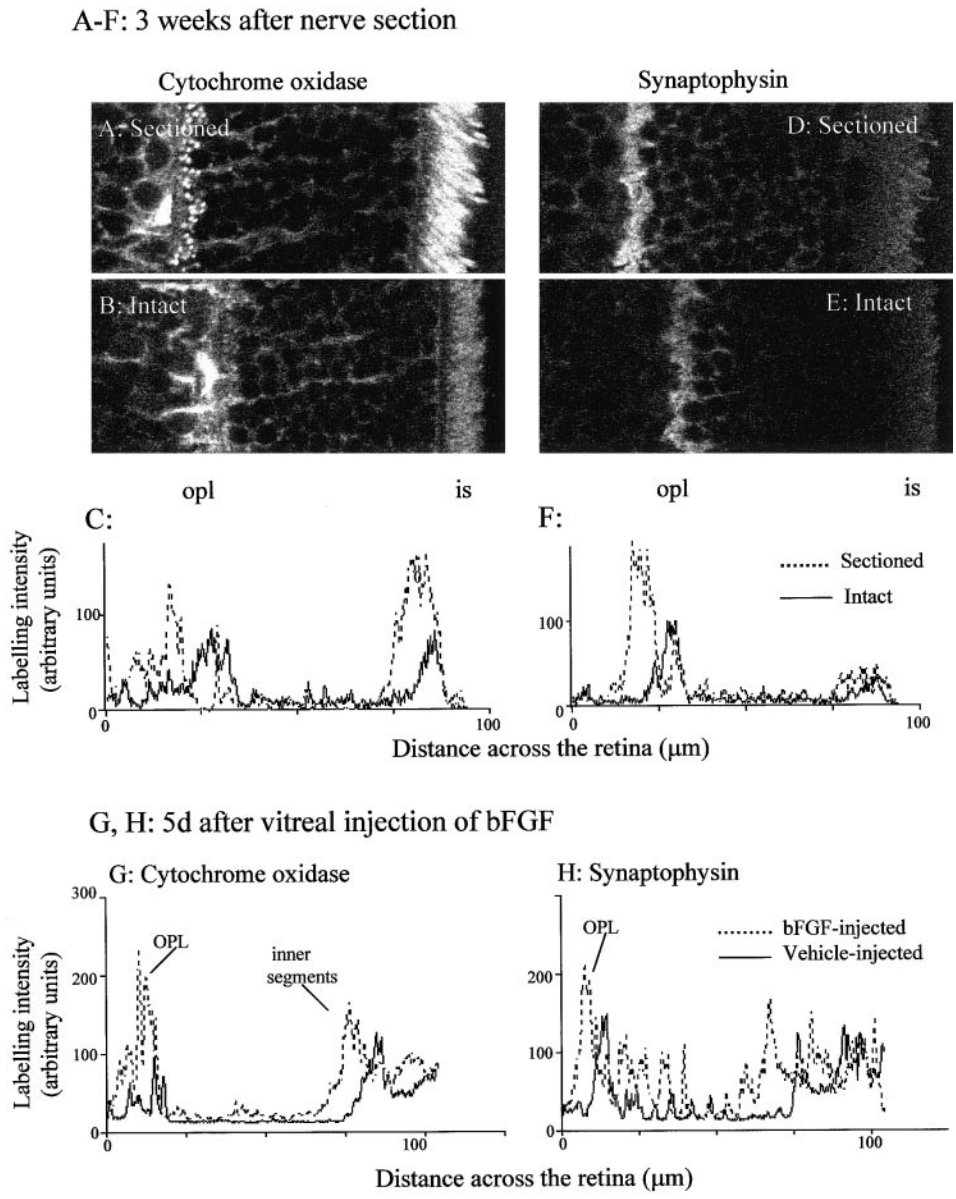


FIGURE 9. Effect of nerve section and bFGF injection on levels of CO and synaptophysin. (**A**) Cytochrome oxidase immunolabeling in nerve-sectioned rat retina 3 weeks after the section. Labeling was strongest in inner segments (is) and strong also in punctate form in the OPL. (**B**) Cytochrome oxidase immunolabeling in nerve-intact retina from the same animal. The same pattern of labeling was found, but signal levels were lower than in the nerve-sectioned retina. (**C**) Labeling intensity for CO measured along lines 9 pixels wide oriented across the retinal layers. The graph has been set in size so that the abscissa approximately matches in extent the sections in (**A**) and (**B**). (**D**, **E**, **F**) The distribution of synaptophysin label was in the same sections as in (**A**, **B**, **C**) and is shown with the same conventions. (**G**, **H**) Labeling intensities for CO and synaptophysin in retinas of vehicle- and bFGF-injected rat retinas 5 days after injection. Conventions are the same as for (**C**) and (**F**).

such as an increase in the stability of bFGF storage in the nerve-sectioned retina or the movement into the neural retina of bFGF generated in nearby structures, such as the retinal pigment epithelium. The regulation of bFGF expression by nerve section follows a slow course, requiring weeks to reach its maximum,[5] so that even a limited increase in storage stability could produce major increase in bFGF protein levels. The abnormal distributions of bFGF protein observed in nerve-intact retinas from some nerve-section experiments were surprising and suggest some loss of bFGF stability in these retinas. The reason for such loss remains unclear, however.

How Does Optic Nerve Damage Affect the ONL?

Bush and Williams[4] were the first to note an effect of nerve section on photoreceptors, reporting that nerve section protects photoreceptors from light damage. Their article and that of Kostyk et al.[5] showed, and the present results have confirmed a centrifugal effect. What is its mechanism?

Three possibilities can be considered. One is that the effect is mediated by centrifugal neural pathways. Such pathways are not prominent in mammals, however, and those that have been described do not reach the ONL.[28] A second possibility is retrograde transmission of a damage signal by the ganglion cells that reaches all regions of the retina. The dendrites of ganglion cells reach no deeper than the IPL, however. In either case, therefore, some further mechanism, such as the spread of the signal through Müller cells, would have to be invoked to account for transmission of the signal to the ONL. There is evidence of such spread, seen in the widespread upregulation in Müller cells of the expression of GFAP protein, after localized retinal damage.[29] The third possibility is that nerve section activates a population of mobile cells, perhaps microglia, which migrate from the site of surgery through the retina, carrying the signal that induces the upregulation of bFGF protein expression in the retina.

Mechanical[10] and light damage,[11,30] laser burns,[7,8] and hypoxia[15] all cause photoreceptor damage, and all also cause an increase of bFGF protein in photoreceptors. When the damage is localized, as with needle-stick and laser burn injuries, the increases in bFGF protein levels is correspondingly localized. The signal released by nerve section may activate the same mechanisms of bFGF concentration.

Is There a Price for Protection?

The present results raise the question of whether the suppression of the b-wave induced by bFGF is tightly linked to the protection also provided by bFGF so that, teleologically, loss of function is the price of protection. As yet, however, evidence is insufficient to answer the question. Understanding of this linkage may be important in understanding the protection provided by trophic factors and their potential value in the treatment of photoreceptor dystrophies.

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