Intravitreal Injection of Fluorochrome-Conjugated Peanut Agglutinin Results in Specific and Reversible Labeling of Mammalian Cones In Vivo

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PURPOSE. To investigate whether intravitreally injected peanut agglutinin (PNA) conjugated with a fluorochrome can specifically label retinal cones in vivo and to evaluate its clinical potential.

METHODS. Fluorescein- or rhodamine-conjugated PNA (0.005%–0.5%) was intravitreally injected into anesthetized mouse, guinea pig, or monkey and retinas were removed at various intervals for fluorescence microscopy. Immunofluorescence and TUNEL assay were carried out to investigate whether PNA injection adversely affected other retinal neurons. Gross visual function was studied in a visual cliff test. The retina of an N-methyl, N-nitrosoarene (MNU)-induced mouse model of retinal degeneration was stained with PNA to evaluate how spatiotemporal pattern of the staining would reflect the progression of degeneration.

RESULTS. Intravitreally injected PNA resulted in specific labeling of cone outer and inner segments and cone pedicles within 30 minutes over the entire retina and in all tested species. The labeling was reversible; cones did not show any labeling 3 weeks after the injection but could be restained with PNA. TUNEL signal and expression pattern of several retinal proteins in PNA-injected mouse retina were indistinguishable from normal. Similarly, visual behavior of mouse 10 hours after the injection was normal. The pattern of PNA labeling in mice with MNU-induced retinal degeneration showed progressive disappearance of cones from the center to the periphery.

CONCLUSIONS. Intravitreal injection of fluorochrome-conjugated PNA results in specific and reversible labeling of mammalian cones in vivo without causing any gross adverse effects. This novel method may eventually provide a clinical tool to examine the patient’s personal and family history and on the simultaneous occurrence of several nonspecific clinical features, such as night blindness, visual field defects, bone-spicule pigmentation, optic disc drusen, macular hemorrhage, and abnormal electroretinogram findings. Genetic tools provide more specific diagnosis but only in a subset of the patients. If the diagnosis is made early, some of the newer treatment strategies, such as stem cell transplantation and retinal prostheses, may prove to be more effective, especially because loss of photoreceptors initiates a series of likely irreversible changes, including remodeling of retinal circuitry.

Various plant lectins are known to bind specifically to different components associated with mammalian, including human, photoreceptors in vitro. For example, peanut agglutinin (PNA), a 110-kDa plant lectin isolated from Arachis hypogea, binds specifically to interphotoreceptor matrix (IPM), which ensheaths cone outer and inner segments and cone pedicles. IPM macromolecules have been cloned and characterized as chondroitin sulfate proteoglycans (IPM-150 and IPM-200). We asked whether fluorochrome-conjugated PNA, when injected intravitreally, would label cones similarly in vivo. If so, it may be possible to visualize cones through the optical path of the eye in a living animal or human using methods such as adaptive optics and scanning laser ophthalmoscopy.

We injected fluorescein- or rhodamine-conjugated PNA intravitreally into mouse, guinea pig, or monkey and found specific and reversible labeling of cone outer segments, inner segments, and cone pedicles. The injection did not cause any overt adverse effects on retinal neurons or gross visual function, as determined by terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL), immunocytochemical localization of various cell-specific retinal proteins and a mitosis marker (Ki67), and Visual Cliff test. To investigate whether the specific cone staining can be used to assess the status of photoreceptor degeneration, we stained retinas of an inducible mouse model of photoreceptor degeneration with PNA and found the spatiotemporal pattern of staining to reflect progressive degeneration. With a more detailed toxicology study and other functional assays, this method of in vivo labeling of cones may become a clinical tool to make a confirmed diagnosis and to assess the extent of damage in some of the retinal degenerative diseases in humans. Part of this report has been published as a meeting abstract.

MATERIALS AND METHODS

Animals and Tissue Preparation

Adult mouse (C57BL/6; n = 50), embryonic day 19 mouse (n = 1), guinea pig (Duncan Hartley; n = 1), and monkey (Macaca mulatta; n = 1) were used in the present study. All experiments were approved by the Institutional Animal Ethics Committee of the National Brain Research Centre or the Committee for the Purpose of Control and
Supervision on Experiments on Animals and conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. The monkey was primarily used for another experiment not related to retina or the visual system, and our experiments were carried out in the anesthetized monkey only after the primary experiment was completed. The animals were anesthetized (mouse, ketamine 100 mg/kg + xylazine 10 mg/kg; guinea pig, ketamine 100 mg/kg + xylazine 30 mg/kg; monkey, ketamine 8 mg/kg + xylazine 0.4 mg/kg). After injecting PNA or N-methyl, N-nitrosourea (MNU; see below), a mouse was killed by cervical dislocation, the guinea pig was killed by anesthetic overdose, and the monkey was perfused transcardially with phosphate-buffered saline (PBS) followed by 4% paraformaldehyde (PFA). Eyes were removed at various time points and hemisected, and the posterior eyecup was fixed in 4% PFA for 1 hour at 4°C. Animals kept for 1 day or more after the intravitreal injection were given gentamicin systemically (5 mg/kg intraperitoneally) and/or as 0.3% eye drops. Retinas were then incised radially and flattened, or they were cryosectioned at 10 mm thickness (model CM3050S; Leica Microsystems GmbH, Wetzlar, Germany) and mounted on a glass slide using mounting medium containing 4’, 6-diamidino-2-phenylindole (DAPI; Vectashield; Vector Laboratories, Burlington, CA). In some cases, to examine whether intravitreally injected PNA produced nonspecific staining in nonretinal tissues, the intact eyeball was fixed with 4% PFA and was sectioned horizontally at 20 minutes, 2 days, or 4 days after the injection. Samples were observed and imaged with an upright fluorescence microscope (Axioplan-2; Carl Zeiss, Gottingen, Germany) or with a confocal microscope (LSM 510 Meta; Carl Zeiss).

Intravitreal Injection of PNA

Two microliters of rhodamine- or fluorescein-conjugated PNA (Vector Laboratories) at a concentration of 0.005%, 0.01%, 0.02%, 0.05%, or 0.5% in PBS was injected and injected intravitreally in mouse, whereas 10 mL and 20 mL of 0.5% PNA was injected in guinea pig and monkey, respectively. A sham control mouse received 2 mL PBS (containing 0.008% sodium azide which was the amount of preservative present in 0.5% PNA). For mouse and guinea pig, a borosilicate glass capillary (outer diameter 1.2 mm; WPI Inc., Sarasota, FL), pulled to an inner tip diameter of approximately 20 mm on a micropipette puller (P97; Flaming-Brown; Sutter Instrument Co., Novato, CA) and connected to a 1-mL syringe by a polyethylene tubing, was used to inject PNA at a point temporal and posterior to the limbus, behind the lens. For monkey, the injection was made using a 30-gauge needle and a standard insulin syringe. The animals were kept for various postinjection intervals (mouse: 20, 30 minutes, 1, 2, 3, 4, 6, 8 hours, 1, 2, 3, 4, 7, 10, 18, 21 days, 1, 3 months; guinea pig and monkey: 30 minutes each), after which the eyes were removed and processed.

Generation of an Inducible Model of Progressive Photoreceptor Degeneration and PNA Staining

MNU is an alkylating agent known to cause progressive photoreceptor degeneration in several species.19-20 We injected a mouse with MNU (60 mg/kg; single intraperitoneal injection; n = 3). Retinas were removed at 6, 24, or 48 hours after the injection and were processed as described above. Flat mounted retinas were incubated in a blocking solution (3% normal horse serum containing 1% bovine serum albumin (BSA) in PBS) for 45 minutes. This was followed by incubation with 0.00125% PNA for 1 hour in a dark, humidified chamber. Retinas were then washed 20 × 10 minutes with PBS, coverslipped, and imaged at a ×5 magnification with the upright fluorescence microscope. Images were later “stitched” together using a software (Photosstitch; Canon, Lake Success, NY) to generate a montage of the entire retina (see Fig. 7).

Immunocytochemistry

Retinal sections were first incubated in a blocking buffer (3% normal horse or goat serum containing 1% BSA and 0.3% Triton X-100 in PBS) in a dark, humidified chamber for 1 hour at room temperature, followed by incubation with a primary antibody overnight at 4°C. The primary antibodies included anti-P7D95 (1:800; Stressgen, Victoria, BC, Canada), anti-syntaxin (1:1500; Stressgen), anti-cPKCa (1:1500; Santa Cruz Biotechnology Inc., Santa Cruz, CA), and anti-nephropro- pylated neurofilament-H (SMI-32, 1:2000; Covance Research Products Inc., Berkeley, CA), all raised in mouse, and anti-Ki67 (NCL-Ki67p, 1:1000; Novocasta, Newcastle, UK) raised in rabbit. The sections were then washed 3 × 5 minutes with PBS and incubated with anti-mouse or anti-rabbit secondary antibody raised in horse or goat and conjugated with Texas Red for 1 hour at room temperature. Sections were washed again for 3 × 5 minutes with PBS, mounted, and imaged with the upright fluorescence microscope or the confocal microscope.

Terminal Deoxynucleotidyl Transferase dUTP Nick-End Labeling (TUNEL)

To detect any PNA-induced apoptotic cell death, TUNEL was carried out according to the manufacturer’s protocol (Roche Applied Science, Mannheim, Germany).21 Briefly, the sections were incubated with permeabilization solution (0.1% Triton X-100, 0.1% sodium citrate, freshly prepared) for 1 minute at 4°C, followed by incubation with TUNEL reaction mixture (1.25:48.75 terminal deoxynucleotidyl transferase:fluorescein-labeled nucleotid mixture; freshly prepared) for 1 hour at 37°C. The sections were then washed 3 × 5 minutes with PBS, mounted, and imaged with the confocal microscope. A positive control section was incubated additionally with DNase I (3000U/mL; Roche Applied Science) after permeabilization, and a negative control section was incubated without the enzyme solution in the reaction mixture.

Visual Cliff Test

A slightly modified visual cliff test was used to test the gross visual ability of the PNA-injected animals.22,23 The cliff test was chosen because it is likely to detect any gross abnormality in the optical or the retinal pathway of the injected eyes. The custom-made setup (see Fig. 6A) consisted an open-top box (75 × 75 × 15 cm) made of clear polymethylmethacrylate glass. The central 30 × 30 cm of the floor was covered from the outside with a black-and-white checkerboard (each square, 1 × 1 cm pattern). The box was raised 1 m above the ground to give the animal a sense of height, and the edges of the central checkerboard created the edge of a “cliff.” Given that the clear base extended beyond the edge of the checkerboard, the cliff was only virtual and the animal did not actually fall. Testing was initiated by placing the animal, with its whiskers trimmed, on the center of the checkerboard, and its behavior was video-recorded (model DCR-HC96E; Sony, Tokyo, Japan) from above for 1 to 2 minutes in bright light (illuminance, approximately 70 µW/cm² or approximately 500 lux; measured with IL1400 photometer; International Lights Inc., Peabody, MA). The videos were analyzed later with a tracking software (AnyMaze; Stoelting Co., Wood Dale, IL) to measure several behavioral parameters, including distance traveled, speed of movement, and amount of time the animal spent on or off the checkerboard. In addition, the entire track of the animal movement was recorded (Fig. 6B).

RESULTS

Intravitreal Injection of Peanut Agglutinin Resulted in Specific Labeling of Cones In Vivo

PNA conjugated with fluorescein or rhodamine, when injected intravitreally in vivo, resulted in specific labeling of cone photoreceptors over the entire retina in all tested species (Figs. 1, 2). Labeling was specific for cones (as revealed by double labeling of PNA and rhodopsin antibody in vitro; not illustrated). The minimum PNA concentration and postinjection duration that consistently produced the labeling in mouse was 0.05% PNA for 30 minutes. Similar to the pattern in fixed retina,7,21-25 the in vivo PNA labeling was specific to cone outer and inner segments and cone pedicles (Fig. 2). In one
In Vivo Labeling of Cones by PNA Was Reversible

For the intravitreal injection of PNA to have a potential clinical use, it is important to know the temporal profile of its binding to the cone IPM in vivo. This may also provide clues about the IPM turnover and about how it is related to the turnover of cone outer segments. To investigate whether the PNA labeling of cones in vivo is reversible, we examined mouse retina at various intervals after intravitreal injection. We found that the PNA labeling was reversible—it disappeared apparently with a periphery-to-center gradient and completely disappeared in 2 weeks.

In Vivo Labeling of Mammalian Cones

Intravitreally injected PNA (0.5%) resulted in specific labeling of cone photoreceptors in mouse (A, B), guinea pig (C, D), and monkey (E, F). Central (left) and peripheral (right) regions. Images are focused at cone pedicles (A–D) or inner segments (E, F) showing their nearly uniform distribution. Scale bar, 10 μm.

**FIGURE 3.** In vivo cone labeling by PNA was reversible. (A) Flat mount of mouse retina removed 1 week after intravitreal PNA-fluorescein injection with a representative area from the midperipheral retina, where cone outer/inner segments (green) are present in a part of the field toward the optic disc (down and to the right, not shown). Cone pedicles (pseudocolored red) are present in the entire area, suggesting that PNA binding of outer/inner segments washes out before that of the pedicles. This image was obtained by superimposing two confocal images at the outer/inner segment level and at the pedicle level. (B) Radial section of mouse retina labeled in vivo by PNA as in (A), showing brightly stained cone outer (arrow) and inner (thin arrow) segments and dimly stained cone pedicles (arrowheads). Scale bar, 10 μm.

**FIGURE 2.** In vivo labeling was specific to cone outer segment, inner segment, and cone pedicle. (A) A flattened mouse retina after intravitreal injection of PNA-fluorescein was imaged twice with a confocal microscope: one (green) at the level of outer (thin arrow)/inner (arrow) segment and the other (pseudocolored red) approximately 25 μm deeper at the level of the cone pedicle (arrowhead). Images were superimposed offline. That outer and inner segments of many cones appear in the same focal plane is a commonly encountered tissue-handling artifact. (B) Radial section of mouse retina labeled in vivo by PNA as in (A), showing brightly stained cone outer (arrow) and inner (thin arrow) segments and dimly stained cone pedicles (arrowheads). Scale bar, 10 μm.

**FIGURE 1.** In vivo labeling of mammalian cones by PNA. Intravitreally injected PNA (0.5%) resulted in specific labeling of cone photoreceptors in mouse (A, B), guinea pig (C, D), and monkey (E, F). Central (left) and peripheral (right) regions. Images are focused at cone pedicles (A–D) or inner segments (E, F) showing their nearly uniform distribution. Scale bar, 10 μm.

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to 3 weeks after injection (data not illustrated). We also found that the labeling of outer/inner segments disappeared before that of cone pedicles (Fig. 3A). To investigate whether the in vivo labeling altered the PNA-binding ability of the IPM, we restained with PNA-fluorescein the retinal sections of a mouse that had been intravitreally injected with PNA-rhodamine 3 months earlier and found specific labeling (Figs. 3B, 3C). We also looked for any nonspecific fluorescence signal in the nonretinal tissues. There was no fluorescence signal in the vitreous, but the lens capsule and inner limiting membrane showed bright fluorescence at 20 minutes, which faded almost completely by 2 days after PNA injection (data not illustrated).

Several Retinal Cell Markers Expressed Normally after Intravitreal PNA Injection

To assess whether the in vivo cone labeling by PNA affected other retinal neurons or synapses, we studied them using specific markers. We immunostained photoreceptor terminals (anti-PSD-95),

rod bipolar cells (anti-cPKCα),

amacrine cells (anti-syntaxin),

and a subset of ganglion cells (anti-nonphosphorylated neurofilament H, SMI-32) at 3 hours, 6 hours, or 3 months after PNA injection and found the staining pattern to be indistinguishable from the normal control (Fig. 4).

In Vivo PNA Injection Did Not Induce Apoptotic Cell Death

We used TUNEL to determine whether PNA caused apoptotic cell death in any of the retinal neurons (Fig. 5). Positive and negative control experiments validated the efficacy of the method (Figs. 5A, 5B). We found that intravitreal injection of PNA did not cause apoptosis in any retinal cell for at least 3 months after injection (Figs. 5C, 5D).

In Vivo PNA Injection Did Not Induce Mitotic Activity in Retinal Cells

Because PNA has been reported to be mitogenic in some tumor cells and normal cells in certain diseases, we explored whether intravitreal injection of PNA induced any mitotic activity in retinal cells. We used Ki67 as a mitosis marker. An embryonic day 19 retina was used as a positive control, which showed Ki67 staining in the outer neuroblastic layer, as expected, whereas an adult retina was used as a negative control (Figs. 6A, 6B). Neither the sham-injected retina at 3 months after injection nor the PNA-injected retinas up to 3 months after injection showed any Ki67 immunoreactivity (Figs. 6C-F), implying that intravitreally injected PNA did not induce mitosis in retinal cells.

PNA-Injected Animals Showed Normal Visual Behavior

A mouse was placed on the central checkerboard and was allowed to move freely in the Visual Cliff apparatus (Fig. 7A). Offline analysis was carried out with video tracking software (AnyMaze; Stoelting). A normal mouse moved at an average speed of 7 ± 6 cm/s (mean ± SD; n = 16) to cover 4.8 ± 3.4 m total distance. The speed on the checkerboard was slightly lower (6 ± 4 cm/s) but was much higher (16 ± 21 cm/s) on the outside area, consistent with our observation that the animal “hesitated” in crossing the virtual cliff to the outside.
When it did venture a short distance outside the checkerboard, it came back quickly (Fig. 7B). It spent 77% ± 1% of the total time on the checkerboard (Fig. 7C). A positive control animal (i.e., a mouse that had lost most of its photoreceptors after 2 days of MNU treatment; see Materials and Methods) was included to test the validity of the analysis protocol. The MNU-treated mouse, as expected, moved frequently outside the checkerboard without hesitating at the cliff edge and spent only 27% ± 1% (n = 4) of the time on the checkerboard (Figs. 7B, 7C). However, the speed of movement of the PBS- or PNA-injected animals was lower during the first 24 hours but increased to normal level by 3 days and remained so for at least 14 days after injection (Fig. 7D), suggesting that even though the PNA-injected animal had normal gross vision as early as a few hours after injection, it moved slowly initially probably because of local pain, leftover anesthetic effect, or both.

![Figure 5](image1.png)

**FIGURE 5.** TUNEL staining showed no apoptosis in retinal cells after intravitreal injection of PNA-rhodamine. (A) Positive control. A normal adult mouse retinal section digested with DNase I, followed by incubation with TUNEL reaction mixture, shows apoptotic signal in all retinal cells. (B) Negative control. A normal adult mouse retinal section incubated with TUNEL reaction mixture, without terminal deoxynucleotidyl transferase enzyme, shows no apoptotic signal. (C, D) Mouse retinal sections taken 30 minutes (C) or 3 months (D) after PNA injection show no apoptotic signal. Sham-injected mice also did not show any apoptosis (not shown). Scale bar, 10 μm.

![Figure 6](image2.png)

**FIGURE 6.** Intravitreal injection of PNA did not induce mitosis in retinal cells, as indicated by Ki67 antibody staining. (A) Positive control (midperipheral mouse retina section at embryonic day 19) showing many Ki67-positive retinal cells in the neuroblastic layer (NBL). (B) Normal adult mouse retina section (negative control) does not show, as expected, any Ki67 immunoreactivity. (C-F) No Ki67-positive cell was detected either at 3 months after sham injection (C) or at 3 hours, 6 hours, or 3 months after PNA-fluorescein injection (D-F, respectively) in the adult mouse. Scale bar, 10 μm.
Spatiotemporal Pattern of PNA Staining in an Animal Model of Retinal Degeneration Showed Progressive Degeneration

A single systemic injection of MNU is known to cause fast and progressive degeneration of photoreceptors from center to periphery. We wanted to study whether the spatiotemporal pattern of the PNA-labeled cones would reflect the status of degeneration in the MNU mouse model. We found that at 6 hours after MNU injection, PNA-labeled cones were present throughout the retina, but at 24 hours and 48 hours after injection, there was progressive decline in the number of cones from the center to the periphery. At 48 hours, only a narrow peripheral rim of PNA-labeled cones remained (Fig. 8).

DISCUSSION

We show here that a single intravitreal injection of PNA conjugated with fluorescein or rhodamine results in specific labeling of cones over the entire retina within 30 minutes in a variety of anesthetized mammals. PNA is known to bind only to cones, which we confirmed by double labeling with antibody and PNA (data not shown). Furthermore, PNA binds to all three cone types. We also show that the labeling is reversible, does not adversely affect the PNA-binding capability of cones, does not cause apoptosis or alter the staining pattern of various retinal proteins, does not induce mitotic activity, and does not impair at least gross visual function. The pattern of PNA labeling has been reported to reflect the progressive retinal degeneration in a genetic animal model. We have confirmed this in an inducible animal model (Fig. 8).

Given that intravitreally injected PNA resulted in cone labeling in all the species tested here, including a nonhuman primate, and that PNA is known to stain cones in postmortem human retina, it is highly likely that it would label the cones in a living human retina. Together these data raise the possibility that this relatively simple method could be eventually used in humans with retinal degenerative diseases. If so, this would allow not only early and confirmed diagnosis but also status assessment of these diseases, which in turn would help in initiating an appropriate therapy in time. Of course, the method must undergo more rigorous toxicology and safety studies before it can be tried in humans. Similarly, a battery of other functional assays, including photopic electroretinography, electrophysiological recording from cones, and physical measurements, would be helpful in detecting any PNA-induced untoward effects.

Various plant lectins conjugated with horseradish peroxidase (HRP), when administered subretinally, have been reported to stain specific photoreceptor components in vivo. However, HRP does not emit, or cannot be excited to emit, light for preferential detection. Rendering HRP visible involves a relatively difficult procedure that can cause clinical complications, including retinal detachment and damage. The method reported here for in vivo labeling of cones has several relative advantages. First, fluorescent tags in the eye can be detected in vivo. Second, fluorescein is commonly used in ophthalmic practice, and other similar fluorescent tags, including indocyanine green, can be possibly tagged to PNA. Third, intravitreal injection of PBS or MNU. Note the reduced speed of movement in both PBS- and PNA-injected animals at 8, 10, 12, and 24 hours after injection, which increased and became comparable to the control animal’s speed from 3 days onward.
real injection is a relatively simple and safe outpatient procedure. Fourth, in vivo binding of intravitreally injected PNA is reversible and does not affect the PNA-binding ability of the cone IPM, suggesting that it may not affect retinal adhesion to the pigment epithelium.\textsuperscript{39,40}

The in vivo labeling of cones may potentially be used in a variety of other investigations, such as those related to cone development, retinal detachment, retinal transplantation, and drug effects (e.g., of toxins or neuroprotectants on cones) in a living human or animal. Our results on the reversibility of PNA staining provide clues about the turnover of IPM, especially the proteoglycans to which PNA binds, and about how this might be related to the turnover of cone outer segments. Two lines of evidence suggest that the turnovers of the IPM and the cone outer segment are independent: PNA labeling completely disappeared in 2 to 3 weeks whereas the outer segment turnover was completed in approximately 10 days.\textsuperscript{41} and the reversal of PNA labeling followed a periphery-to-center gradient whereas outer segment phagocytosis in the center occurs at higher rate than in the periphery.\textsuperscript{42} These findings are consistent with the idea that IPM turnover occurs through nonphagocytic endocytosis.\textsuperscript{43,44}

Various genetic manipulation, retrograde transport, and viral vector-based methods have also been used to label different retinal cells\textsuperscript{16,17,45–47} but may not be readily applicable to humans. Moreover, many of the viral vectors may not cross the inner limiting membrane in vivo. It is unclear which biophysical properties of retina allow or disallow penetration by an exogenous molecule of a given size.\textsuperscript{48,49} The PNA, even though it is a relatively large (110 kDa) molecule, can evidently cross the inner limiting membrane to bind specifically to the cone IPM.

Recent advancements in optical methods, such as adaptive optics and confocal scanning laser ophthalmoscopy, allow identification of single cells, including photoreceptors in vivo.\textsuperscript{14–17} The method of in vivo labeling of cones with fluorescent PNA offers a significant complement to these advancements in visualizing cones in a living human or animal retina.\textsuperscript{17} One potential problem here is related to axial resolution of the optical method to resolve cone pedicles from outer and inner segments. Another may be background noise if some of the injected PNA binds to nonretinal tissues. We did not find any signal in the vitreous, but nonspecific fluorescence in the lens capsule and inner limiting membrane in the initial period after PNA injection should be considered. In one retina, we noticed fluorescence in the IPL, which has also been reported in some cases of fixed retina,\textsuperscript{7} but it is not clear what causes occasional IPL labeling. In a separate experiment we found that a high concentration (≥0.05%) of uncentrifuged PNA tended to produce bright punctate staining in the IPL of fixed retinal sections (data not shown). It is possible that the IPL staining after intravitreal injection of PNA observed in one retina resulted from inadequate centrifugation or inadvertently fast bolus injection. In all other cases, however, there was no discernible staining in the IPL, and the signal-to-noise ratio of the specific labeling was considerably higher than that in vitro (data not shown), probably because of the prolonged “natural washout” of the nonspecific binding.

In summary, we report here a novel method of labeling mammalian cones in vivo by intravitreal injection of fluorochrome-conjugated PNA. This should significantly complement the existing optical methods of visualizing single cones in a living human or animal by enhancing the cell’s optical contrast. Although more experiments may be required to establish the safety of this method for human application, we show in a series of experiments that PNA injection does not cause gross morphologic or functional adverse effects.

\textbf{Acknowledgments} 

The authors thank Neeraj Jain for providing the anesthetized monkey; Shyamala Mani for Ki67 antibody; and David Williams, William Mergan, John Flannery, and Melanie Campbell for useful discussions.
References