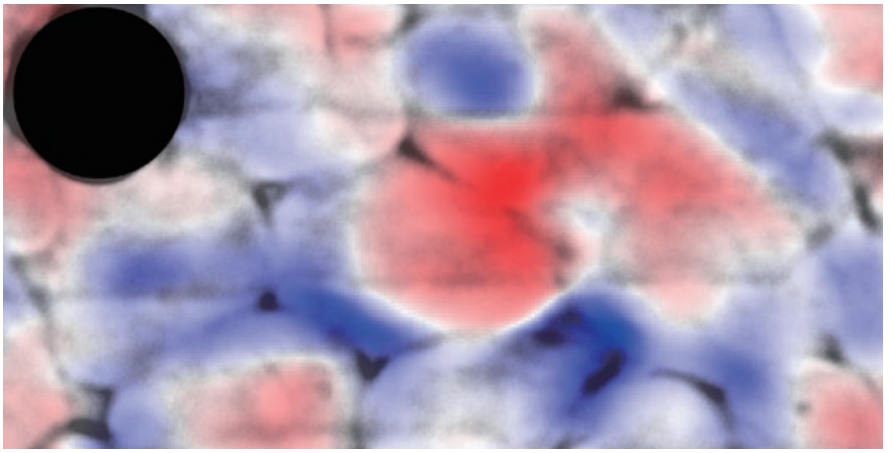
IMAGING

Watching Nerve Cells Deform as They Fire



IN VITRO. *Color overlay of firing nerve cells shows membrane deformation at the peak of the action potential. (Gray = nerve cells; red = movement toward viewer; blue = movement away from viewer; black dot = opaque electrode.)*

Palanker Laboratory, Stanford University

CALIFORNIA SCIENTISTS HAVE DEVEL-oped a noninvasive method to detect nanometer-scale changes in the shape of human nerve cells as they fire, a development that could someday enable ophthalmic researchers to assess and quantify the eye's neural functioning at the cellular level.

Using an interferometric microscope and a high-speed camera that imaged in vitro cells at up to 50,000 frames per second, the researchers assembled videos showing the membranes rounding slightly as they fired, then returning to normal.[1]

The genetically altered human cell line used in their experiments, called HEK-293, was chosen because it has regular, spontaneous electrical spikes. To separate the minuscule deformations from noise in the data, the scientists combined 50 frames at a time, averaged each pixel to strengthen the signal, and then used a self-reinforcing algorithm to boost the signal further.

In this way, they determined that the cells' outer dimensions changed by between 1 and 3 nm, fluctuating as the action potentials propagated across the cells. These surrogate optical measurements of electrical activity corresponded precisely to the signals detected conventionally with electrodes placed near the cells.

"This nanometer-scale shape change is very difficult to see—but with ultrafast quantitative phase imaging, it actually turns out to be visible," said Daniel Palanker, PhD, who led the investigation.

Advantage: noninvasive. The technique's major potential advantage compared to existing methods of measuring in vivo neuronal activity in the eye is that it is noncontact and noninvasive, said study coauthor Kevin C. Boyle, MS, a PhD student in Dr. Palanker's laboratory at Stanford University in Palo Alto, California.

"Nothing needs to be added to the cells—no fluorescent dyes, no optogenetic viruses, no markers, no additional preparation. It's all done optically," Mr. Boyle said. "It's also high throughput. You're getting much more information about what's happening across an individual cell and also across multiple cells in a field of view."

Deformation of nerve cells when they fire was first described decades ago, based on observations of large nerves from crustaceans, he said. "But no one has been able to see the real thing in mammalian cells because the deformations are much smaller," Mr. Boyle said.

But why do the membranes deform at all? "Based on our current hypothesis, which is from a model developed by others who have studied this effect, we believe that when the action potential happens the electrical potential generated across the cell membrane changes the membrane tension. This change tends to minimize the surface area of the cell membrane, causing the cell to become more spherical during the action potential," he said.

Ultimate goal. The NEI views better imaging as essential for the advancement of regenerative therapies for retinal diseases, and it is funding five such projects through its Audacious Goals Initiative. This new technique, along with adaptive optics and optical coherence tomography, may eventually be used to build a device to noninvasively assess the electrical activity of the optic nerve and retinal cells.

—Linda Roach

1 Ling T et al. *Light Sci Appl.* 2018;7:107.

Relevant financial disclosures—Mr. Boyle and Dr. Palanker: None.

DRUG SAFETY

Fungal Outbreak Posed Difficult Treatment Issues

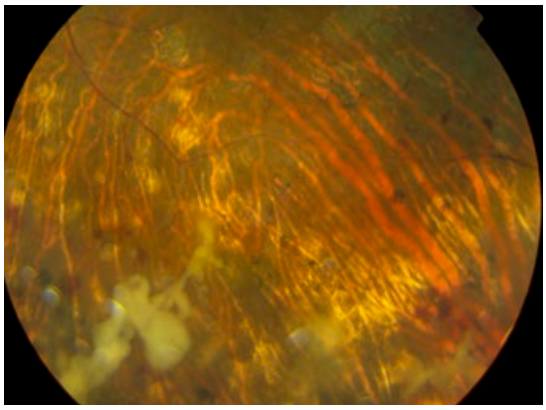
SEVEN YEARS AFTER AN OUTBREAK of fungal endophthalmitis from contaminated triamcinolone, ophthalmologists whose patients were infected have reached some sobering conclusions about the difficulties of treating such cases.

In 2012, 30 eyes nationwide were infected with a plant fungus, *Bipolaris hawaiiensis,* from intravitreal injections of contaminated triamcinolone.[1,2] The drug had been compounded by the now-defunct Franck's Compounding Pharmacy in Ocala, Florida.

Five-year outcomes. Ophthalmologists who treated 23 of these patients (25 eyes) in Los Angeles and New York City have now reported their five-year follow-up outcomes.[3]

Treatment challenges. The outbreak started as an acute crisis but evolved into a chronic, puzzling management problem, the retrospective chart review revealed. For example:

- Some infections presented as late as 10 months postinjection.
- Despite appearing sterile after treatment, eyes that were enucleated still had hyphae present. (Because the organism is difficult to culture, the hyphae's viability could not be determined, the authors reported.)
- Intravitreal antifungal injections, vitreous tap, and pars plana vitrectomy did not resolve the infections. Nor did a standard, on-label systemic regimen for treating fungal infections (200 mg oral voriconazole twice a day for six weeks). "With all the patients, as soon as the oral voriconazole was stopped after six



PRESENTATION. *This patient's visual acuity was 20/50 at onset of fungal endophthalmitis. He eventually underwent enucleation.*

GLAUCOMA

Asymmetric Pattern of VF Loss Found in POAG

RESEARCHERS WHO PREVIOUSLY FOUND THAT PRIMARY open-angle glaucoma (POAG) and primary angle-closure glaucoma (PACG) have different patterns of visual field damage[1] now report yet another difference between the two glaucomas. An asymmetric rate of visual field (VF) loss seems to be a feature of eyes with POAG—but not those eyes with PACG.[2]

"This difference further promotes our understanding of mechanisms of visual field loss underlying both glaucoma types," said Ryo Asaoka, MD, at the University of Tokyo in Japan.

Study goal. The researchers set out to determine and compare global, region-wise, and point-wise rates of VF loss in POAG and PACG eyes, with the goal of identifying whether POAG and PACG eyes progress at different rates and/or with different patterns.

To do so, they reviewed the medical records of 282 patients (440 eyes) with POAG and 49 patients (79 eyes) with PACG who were treated at two university hospitals in Japan between 1998 and 2016. All had at least six reliable visual field tests with Humphrey Field Analyzer II. Glaucoma was the only disease that caused VF damage.

Asymmetric findings. In POAG, the rate of VF loss was faster in the superior hemifield compared to the inferior hemifield, particularly in the central, paracentral, and peripheral arcuate 2 regions. This asymmetry was not observed in PACG eyes. "This was not necessarily surprising because we already knew there were considerably different patterns in visual field damage between POAG and PACG," Dr. Asaoka commented.

In a separate finding, PACG eyes had a consistently faster global rate of VF loss compared to POAG eyes; however, this difference was not statistically significant.

Questions remain. Dr. Asaoka wants to better understand the disease mechanisms related to VF loss and how they might differ between POAG and PACG eyes.

For example, VF loss in PACG appears to be purely due to an elevated IOP, he said, whereas loss in POAG is more complex. Another possible contributing factor is corneal hysteresis; this "is very closely associated with the progression of glaucoma in POAG," he said. In a separate study, Dr. Asaoka and his colleagues confirmed that concept in a Japanese population with a very high prevalence of normal-tension glaucoma,[3] and they plan to continue to look at these contributing factors, he said.

In the clinic. Dr. Asaoka advised clinicians to consider that superior VF is likely to progress faster in POAG, whereas both superior and inferior hemifields can progress relatively quickly in PACG. *—Miriam Karmel*

1 Yousefi S et al. *Invest Ophthalmol Vis Sci.* 2018;59(3):1279-1287.
2 Yousefi S et al. *Invest Ophthalmol Vis Sci.* 2018;59(15):5717-5725.
3 Matsuura M et al. *Sci Rep.* 2017;7:40798.

Relevant financial disclosures—Dr. Asaoka: None.

weeks, the infection came back with a vengeance," said lead author Kent W. Small, MD, who practices in Los Angeles and Glendale, California.

- Patients required prolonged systemic off-label, high-dose treatment with oral voriconazole (300 mg twice daily for six to 12 months) to eliminate clinical signs of infection.
- Despite apparently successful treatment, some of the eyes continued to deteriorate, most frequently from hypotony. Only eight of the 25 eyes had final visual acuity (VA) of 20/50 or better. Five eyes had to be enucleated, and the VA in an additional five eyes was light perception or no light perception.

Need for prompt communication. In addition to such clinical lessons, the endophthalmitis outbreak was an example of the importance of the need for meticulous oversight of compounding (and other) pharmacies as well as the importance of professional transparency among ophthalmologists when an outbreak occurs, Dr. Small said.

In Dr. Small's own practice, 17 eyes were infected with *B. hawaiiensis*. "No practice—private, academic, or governmental—is immune to receiving contaminated medication from any pharmacy. But when this sort of incident happens, a feeling of isolation is overwhelming because you realize you are on your own in unchartered waters," Dr. Small said. After his initial feelings of dismay and embarassment, he said, "I soon realized I did nothing wrong. There is nothing I could have done differently to have prevented this."

He concluded, "The ophthalmic community needs to know about these kinds of incidents. We need to alert each other and learn from each other how to handle them." *—Linda Roach*

1 Mikosz CA et al., and the Fungal Endophthalmitis Outbreak Response Team. *Emerg Infect Dis.* 2014;20(2):248-256.

2 MMWR *Morb Mortal Wkly Rep.* 2012;61(17):310-311.

3 Small KW et al. *Ophthalmol Retina.* 2019;3(2):133-139.

Relevant financial disclosures—Dr. Small: None.

RETINA

After Anti-VEGF: When Patients Don't Return

RESEARCHERS AT WILLS EYE HOSPITAL have added to their growing body of evidence that too many patients are lost to follow-up after receiving an anti-VEGF injection.

In their most recent study of nonadherence, one-fourth of patients with nonproliferative diabetic retinopathy (DR) and diabetic macular edema (DME) did not return for follow-up within 12 months after receiving an injection.[1] Hispanics, lower income patients, and those with poorer baseline vision were among those most likely to be lost to follow-up.

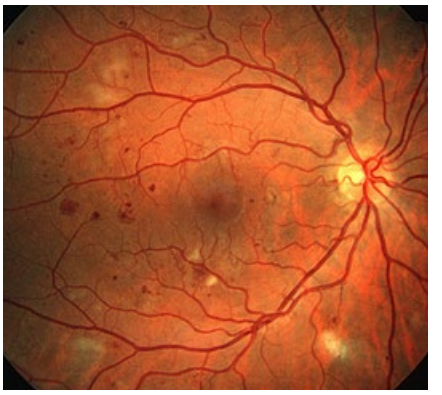
Recalcitrant problem. This finding was consistent with the group's previous studies of patients receiving anti-VEGF injections for wet AMD, retinal vein occlusion, and proliferative DR.[2]

"Almost across the board, with all diagnoses we have looked at, about a quarter of patients are lost to follow-up immediately after receiving an anti-VEGF injection," said Jason Hsu, MD, at Wills Eye Hospital in Philadelphia. "Given the importance of ongoing therapy to prevent vision loss, these real-world findings are of significant concern."

Parsing risk factors. In this retrospective cohort study, 1,632 patients received a total of 10,884 anti-VEGF injections over 15,803 clinical visits. Of these patients, 355 had no further visits for more than 12 months after the last injection.

The researchers also found the following:

- By self-identified racial group, 35% of Hispanic patients were lost to follow-up, followed by 30.6% of Asian patients, 29.1% of black patients, and 21.3% of white patients.
- Patients living in zip codes with lower-than-average adjusted gross income were more likely to miss the next appointment. For instance, 32.4% of those in a low-income zip code (defined as less than $50,000 per year) were lost to follow-up. In contrast, 18.4% of those in a higher-income zip code (defined as more than $75,000 per year) did not return for treatment.
- Decreasing baseline vision also was significantly associated with risk of nonadherence. In a subgroup of the 923 DME patients, the lowest rate of attrition (12.4%) was found in those with a baseline VA that was 20/50 or better; the highest rate of attrition (32.5%) occurred in those with a baseline VA of 20/80 or worse.
- The patient's stage of nonproliferative DR did not significantly predict the risk of loss to follow-up or interact with other factors.



***NONCOMPLIANCE**. Approximately 1 in 4 patients with nonproliferative DR (shown here) and macular edema had no follow-up visit for at least a year postinjection.*

Clinical implications. Dr. Hsu urged physicians to track patients carefully. He also suggested making phone calls or sending letters to encourage patients to return for care. "I worry that many patients with preventable vision loss are losing their sight as a result of nonadherence with follow-up." (See also page 22.) *—Miriam Karmel*

1 Gao X et al. *Ophthalmol Retina.* 2019;3(3):230-236.

2 Obeid A et al. *Ophthalmology.* Published online Aug. 2, 2018.

Relevant financial disclosures—Dr. Hsu: Genentech/Roche: S.

See the financial disclosure key, page 13. **For full disclosures,** including category descriptions, view this News in Review at aao.org/eyenet.