

used." He noted, though, that because they are a physics lab testing a new technique, the scientists looked at only truncated proteins. The findings must be followed up with a study of full-length proteins.

Indeed, the researchers are beginning to look at such proteins. "We are starting to address the biological part of the question," Müller added. They also are working to refine the technique itself. Currently, determining the stoichiometry of

a complex requires more than one measurement. The team hopes to resolve the composition of a complex directly using a more powerful analysis tool; this will require further research, Müller said. □

Gary Boas

Optical brain imaging reveals development of mouse binocular visual cortex

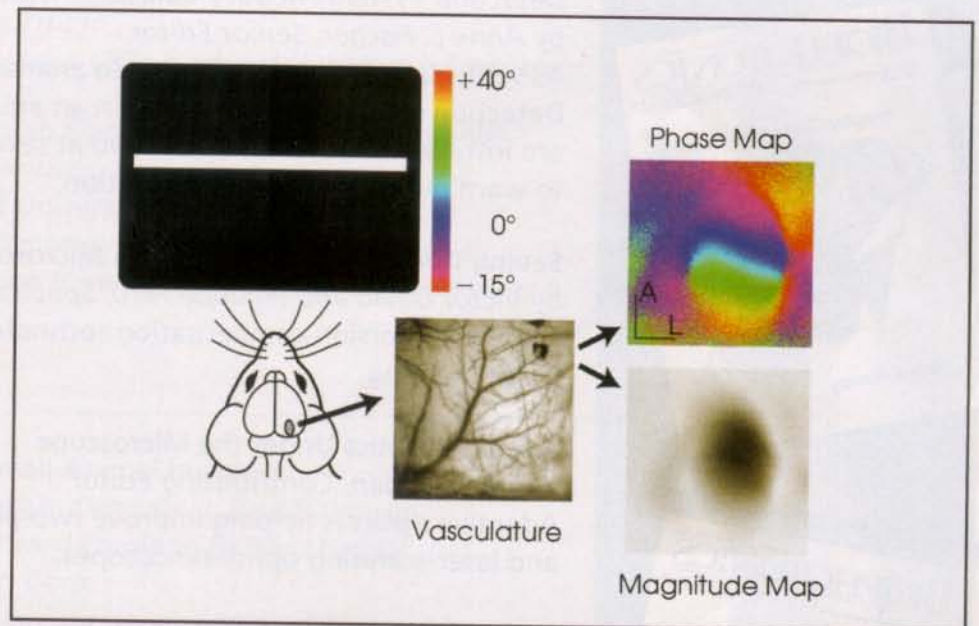
Work may help people with problems in one eye

Optical imaging research into how the binocular visual cortex develops in mice has led Spencer L. Smith and Joshua T. Trachtenberg of the University of California, Los Angeles, to question a commonly held theory of brain development. The work, which relies on intrinsic signal optical imaging, demonstrates that the brain circuits that control binocular vision are not only plastic but also sensitive to noise. The research may eventually have implications for treating people who suffer from amblyopia, commonly known as lazy eye.

Binocular visual space is the area in front of an animal that can be seen by both eyes, and the binocular visual cortex is the part of the brain surface that processes vision for that area. The left part of the brain takes care of the right half of the binocular space and vice versa. Both eyes can see light in this region, so either can create a neuronal response. However, occluding an eye for a period of time causes the binocular visual cortex to become more responsive to vision from the still-open eye. This is called ocular dominance plasticity.

In previous work, other scientists demonstrated that ocular dominance plasticity is most robust during a short developmental window, typically a week or more after an animal's eyes first open. "Eye occlusions before or after this 'critical period' resulted in little or no changes in physiology," Smith said. "This led to the belief that the brain circuits involved were not plastic until this brief period of time."

To understand the development of this area of the brain, Smith and Trachtenberg conducted a series of brain imaging studies on normal mice, on mice that had one eyelid permanently shut so that it never opened normally and on a third group that had an eye surgically removed before the "critical period."



Using intrinsic optical imaging, researchers have determined that, in animals with binocular vision, the brain relies on visual input from the left eye to develop the retinotopic map for the right eye and vice versa. They imaged the visual cortex through the skull using 700-nm light. The animal views a white horizontal bar drifting up (or down) at 0.125 Hz while an 8-min video is recorded by the camera. Using Fourier analysis, the investigators transform the time series of each pixel location in the movie. Then, they combine the magnitude and phase of each pixel's oscillation at 0.125 Hz to make two maps. The magnitude map reveals the location of the visual cortex and the strength of its response, and the phase map shows the phase relationship between the stimulus and the response at each pixel, thus giving a measure of retinotopy.

They conducted imaging studies on the three groups periodically during the critical period, beginning 13 days after the mice were born and continuing until day 23. The work is detailed in the March issue of *Nature Neuroscience*.

They used intrinsic optical imaging because it detects metabolic changes that are associated with neural activity, Smith explained. Likely, several physiological mechanisms contribute to the signal, including deoxygenation of blood, changes in capillary diameter and cell swelling. "These signals are well-correlated with

neural activity," he said.

After resecting the scalps of live anesthetized mice, the researchers imaged through the thin bone of the skull. They shone a light source from Volpi USA of Auburn, N.Y., that was filtered for 700 nm onto the revealed brains and measured the reflectance. High neural activity reduces the reflectance.

Because they used Fourier analysis, they used a fast, high-well-depth, 12-bit camera from Dalsa of Waterloo, Ontario, Canada.

Intrinsic signals can be detected only

through averaging with slower cameras. For low-magnification, high-numerical-aperture imaging, they fitted the camera with a tandem-lens microscope: two Nikon F-mount lenses — a 35-mm, $f/1.4$ and a 135-mm, $f/2.8$ — facing each other with the 35-mm lens closest to the animal. They used 700-nm bandpass filters from Thorlabs and CVI Laser at the light source and at the camera. For image collection, they used a Matrox frame grabber and custom-developed software.

In normal mice, the team discovered that between day 13 and day 18, the cortical retinotopic map of the ipsilateral eye developed more slowly than the maps for the contralateral eyes. By day 17, however, the maps for each eye stabilized and did not develop further.

For the mice that had one eye kept shut, the visual development was very different. By day 15, blocking vision in the contralateral eye caused a significantly less organized cortical map. Although the contralateral maps improved over time, they remained much less organized than those of normal mice. Interestingly, depriving one eye of normal vision impeded the development of the uncovered ipsilateral eye, even at day 17 when development would be expected to be near normal. This ability of the covered eye to retard development of the maps of the normal uncovered eye indicates that binocular competition takes place even before the critical period.

However, for mice that had one eye surgically removed, the story is different.

In contrast to the cortical maps in mice with two normal eyes, one of which was deprived of normal vision, the cortical maps in mice with only one eye developed much more rapidly. In fact, at day 15, these mice had maps that were much more developed than those in mice with normal vision in both eyes.

The investigators think that patterned vision is the real key to normal visual development. "We use the term 'patterned vision' to differentiate it from the remaining vision when an eyelid is closed. Diffuse light can still make it through the eyelid and cause neural activity," Smith explained. This neural activity creates "noise" in the system and makes the organization of the cortex much more difficult. Removing an eye completely, or simply chemically stopping its electrical activity, reduces the noise and accelerates the development of the ipsilateral eye's map.

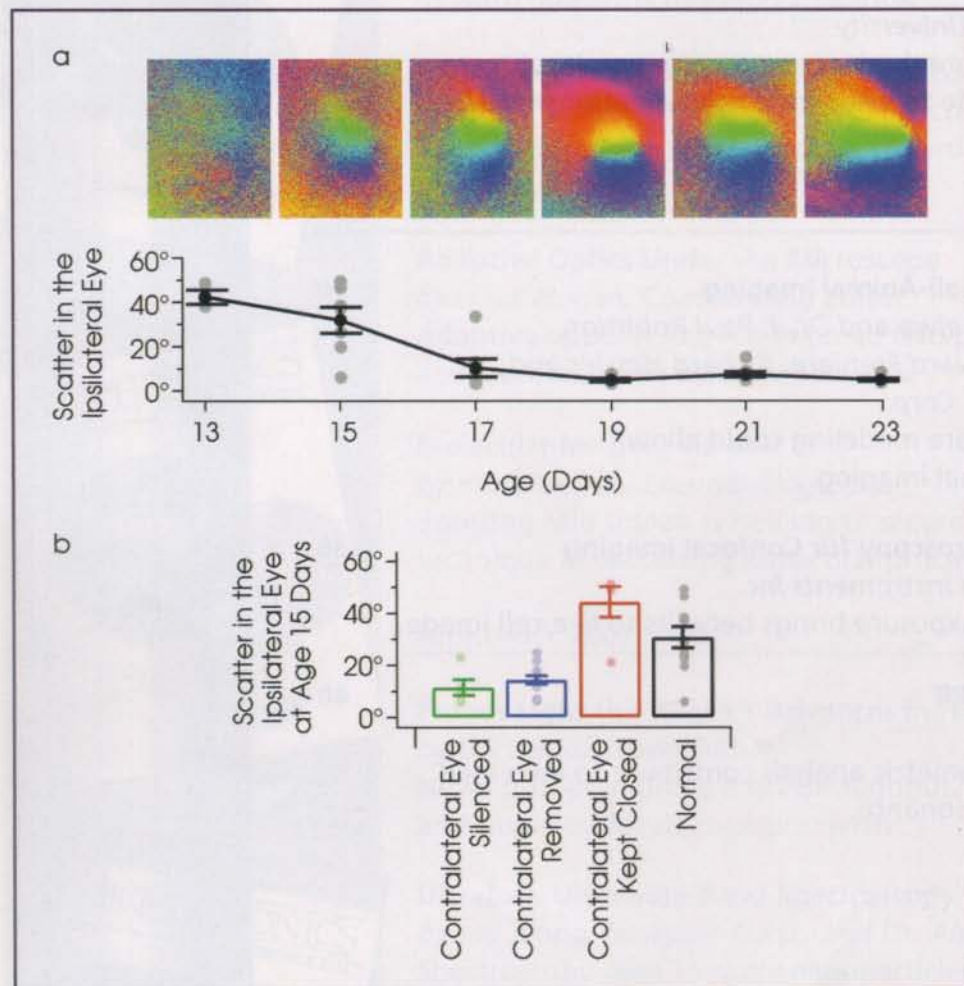
"Noisy vision retards the development not only of the closed eye's brain circuits but, surprisingly, also of the open eye's circuits," Smith added.

He explained that the research suggests that a well-known treatment for one type of amblyopia may be a poor strategy. Anisometropic amblyopia occurs when a person has two functioning eyes, but the eyes have substantially different resolving powers. For example, one may be nearsighted and the other farsighted. One common treatment for this condition is to obscure the vision in the good eye with medicine or with a patch, supposedly pushing the weak eye to strengthen itself.

The technique, however, may actually retard recovery of the weak eye, because it tries to set up its cortical circuitry in the presence of a strong, but noisy, dominant eye. "A better strategy would be to simply give the patients corrective lenses. Our results suggest that the weak eye will recover on its own [when] its vision matches that of the dominant eye," Smith said. A large multicenter study reported in the June 2006 issue of *Ophthalmology* found that this was indeed the case: Patients with only glasses and no patch recovered fine.

From here, the scientists plan to use the same technique to study cortical plasticity in various-age animals. They also are using other techniques, such as chronic imaging using multiphoton microscopy, to study plasticity at the cellular level. □

Kevin Robinson



After the mouse's eyes open about 11 days after birth, the ipsilateral eye retinotopic map gradually becomes more refined until it is well-organized by day 19 (a). If the contralateral eye is removed or pharmacologically silenced, the ipsilateral eye's retinotopic map develops more quickly than normal. However, if the contralateral eye is kept closed, the development of the ipsilateral eye's retinotopic map is retarded. This likely is caused by noisy activity generated in the contralateral eye as it receives diffuse light through the eyelid (b).