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RESEARCH NEWS

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Tracing the Neural Circuitry of 'Second Sight'

Researchers have traced the light-sensing circuitry for a type of "second sight" that is distinct from the conventional visual system and seems to interact directly with the body's internal clock. The researchers speculate that subtle genetic malfunctions of this machinery might underlie some sleep disorders.

In an article published in the February 8, 2002, *Science*, a research team led by Howard Hughes Medical Institute investigator King-Wai Yau described the circuitry, which consists of a subset of nerve cells that carry visual signals from the eye to the brain. The scientists showed that circadian-pacemaker nerve cells almost certainly depend on a different light-sensing pigment, called melanopsin, than the conventional visual system, which relies on rod and cone photoreceptors arrayed across the retina.

Biological, or circadian, clocks operate on a 24-hour cycle that governs sleeping and waking, rest and activity, body temperature, cardiac output, oxygen consumption and endocrine gland secretion. In mammals, the internal circadian clock resides in the brain, and sunlight is the cue that resets this clock daily.

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> **- King-Wai Yau**

Basic insights into the circadian system could lead to improved treatment for such problems as jet lag and depression, and even help optimize drug treatments affected by the rhythmic changes in body hormones.

"It's been known for twenty years that the eyes are required to set the circadian clock, a process called photoentrainment," said Yau, who is at The

Johns Hopkins University School of Medicine. "But over the last few years or so, increasing evidence has suggested that the retinal rods and cones are not the only receptors involved in light detection." For example, studies showed that mice genetically altered to lack functioning rods and cones still showed photoentrainment of their circadian clocks, said Yau. The same non-visual, light-sensing system also appears to govern the pupillary light reflex, the process by which the pupil opens or closes in response to changes in light intensity.

Previous studies suggested that this "second sight" system consists of neural circuitry that connects the retina to the brain's circadian control center, called the suprachiasmatic nucleus (SCN), said Yau.

A key discovery, said Yau, was the identification of a candidate circadian light-sensing pigment, melanopsin, from the retina by Ignacio Provencio and his colleagues at the Uniformed Services University. Yau and his colleagues sought to map the circuitry of melanopsin-containing nerve cells from the retina to the brain. Visual signals are carried to the brain by retinal ganglion cells.

The scientists used fluorescence-labeled antibodies that selectively attached to melanopsin to label the retinal ganglion cells that might be involved in circadian photoentrainment. "We got a very clear result," said Yau. "Of the approximately 100,000 retinal ganglion cells, only about 2,500, or two percent, were labeled by the melanopsin antibody." Labeling revealed that melanopsin is present throughout those cells -- including the cell bodies, the axons and the finely branching dendrites, said Yau.

To map the connections of these cells to the brain, the scientists created a "knock-in" mouse in which each melanopsin-expressing cell also carried a marker gene called tau-lacZ, whose coded protein could be selectively stained. This protein was capable of traveling down the axons of the ganglion cells, revealing their targets.

"The labeling was just beautiful," said Yau. "It showed that the axonal projections of these cells reach out to innervate the SCN in a very dense manner.” The axons also project to other areas of the brain such as the intergeniculate leaflet, which in turn connects back to the SCN, where the main circadian pacemaker is located. Thus, there are feedback-control loops in photoentrainment that involve multiple structures of the brain. In addition, the mapping showed that the melanopsin-containing ganglion cells innervated the brain region known to control the pupillary reflex.

A major question was whether the melanopsin-expressing retinal ganglion cells are truly intrinsically sensitive to light. In an accompanying paper in the same issue of Science, David Berson and colleagues at Brown University report used pharmacological blockers to show that the retinal ganglion cells projecting to the SCN are, indeed, photosensitive by themselves. Berson, who also collaborated on Yau’s *Science* article, did key experiments to show that intrinsic photosensitivity and the expression of melanopsin showed strong one-to-one correlation in the retinal ganglion cells.

According to Yau, the discovery of the architecture, circuitry and photosensitivity of the melanopsin-containing retinal ganglion cells is an important step understanding how the circadian system is controlled.

Yau and his colleagues are planning more studies, such as to explore the behavioral effects of knocking out photoentrainment. They would also like to learn more about the system's neural wiring, how it detects light and how it wires itself during embryonic development. These types of studies could improve understanding of both the system itself and how it might malfunction to cause sleep disorders, said Yau.

"Sleep disorders are a major health problem that profoundly impact the lives of those affected," he said. "As we find out more about the circadian photoentrainment system, we may well discover subtle genetic alterations that cause such disorders in a previously unsuspected way."