

JANUARY 10, 2003

## Molecule Helps Pupils Respond to Light

Researchers are reporting progress in understanding whether a second light-sensing pathway in mammals indeed contributes to the detection of ambient light for controlling body functions.

In an article published in the January 10, 2003, issue of the journal *Science*, the researchers report that the molecule melanopsin is necessary in order for the pupil to constrict properly in response to light, a function termed the pupillary light reflex.

The latest findings by Howard Hughes Medical Institute investigator King-Wai Yau at Johns Hopkins University and his colleagues from Imperial College in London and Brown University build on studies they published last year in which they traced the neural circuitry for this newly discovered light-sensing pathway that is distinct from the primary visual pathway.

---

"For us, the most important question was whether this melanopsin pathway is of any physiological importance."

- King-Wai Yau

---

In those studies, Yau and his colleagues showed that the neural circuitry is constructed of a small subset of intrinsically photosensitive retinal ganglion cells (RGCs) that carry visual signals from the eye to the brain. These RGCs project specifically to brain centers involved in circadian-pacemaker activity and the pupillary light reflex, accessory visual functions that do not require image-formation on the retina. Biological, or circadian, clocks operate on a roughly 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.

Improved understanding of the circadian system could lead to better treatments for jet lag and depression, and may help optimize drug treatments

affected by changes in hormone levels.

Although earlier studies had indicated that melanopsin was part of a light-sensing system, in the latest research Yau and his colleagues sought to demonstrate that the molecule is indeed required for the light-sensing ability of this system and that the system has a true physiological function.

They first developed a knockout mouse in which they completely replaced the melanopsin gene with a tracer gene. In initial studies, they found that although knocking out the melanopsin gene did not affect the genesis and wiring of the specific RGCs responsible for the light-sensing pathway, it did make the RGCs unresponsive to light.

Determining that in such animals these specific retinal ganglion cells were still present but they became light-insensitive was crucial, because it told us, first, melanopsin is indeed required in order for these cells to be intrinsically light-responsive and, second, that whatever functional defect we found in the animal could be directly attributed to the loss of photosensitivity of these retinal ganglion cells rather than to elusive causes such as mis-wiring in the circuitry, said Yau.

To determine the physiological effect of the melanopsin-deficient cells, the researchers chose to measure how the pupils of the knockout mice constricted in response to a gradually increasing intensity of light, because this reflex is fast, precise and can be readily be quantified.

In a normal animal, increasing the light intensity would progressively increase the constriction of the pupil, until it is no more than a pinhole, said Yau. But in the knockout animals, while the pupil begins to constrict normally in dim light, at higher intensities of light the reflex seems to hang. That is, the pupil never constricts down to the same small size as in the normal mouse.

Since the knockout mice still exhibited some pupillary light reflex, albeit diminished, Yau and his colleagues suspected that the melanopsin-dependent reflex might be complemented by the rods and cones, the photoreceptors for the conventional, image-forming visual pathway. Thus, they tested the pupillary reflex in another strain of mouse that have lost the rods and cones due to degeneration.

We found in these mice that the threshold of the pupil reflex is elevated tremendously, said Yau. However, as you increase the light intensity, eventually the pupils start to constrict; and at high intensities, it constricts to the normal level. Thus, the pupillary light reflex involves two complementary mechanisms, one being the rod/cone system, and the other being the melanopsin-associated system.

There is overlap between the two systems, said Yau. The rods and cones are responsible for the high sensitivity of the reflex, but they cannot complete the job, said Yau. On the other hand, while the melanopsin system is not highly sensitive to light, it alone can nonetheless bring the reflex to completion.

Could there be yet a third mechanism that aids in the reflex? Yau said that his groups analysis of the characteristics of the two mechanisms suggests that a third mechanism would have a negligible effect, if it exists at all. In further studies, they plan to produce a mouse lacking both the rod/cone system and the melanopsin-dependent system, to determine whether the mice would lack the pupillary light reflex completely.

For us, the most important question was whether this melanopsin pathway is of any physiological importance, said Yau. Now we have shown that it is, based on the simple pupil reflex. The next step will be to examine closely other, more complex physiological functions, such as circadian photoentrainment.