11. Dell'agnello, C., Leo, S., Agostino, A., Szabadkai, G., Tiveron, C., Zulian, A., Prelle, A., Roubertoux, P., Rizzuto, R., and Zeviani, M. (2007). Increased longevity and refractoriness to Ca(2+)-dependent neurodegeneration in Surf1 knockout mice. Hum. Mol. Genet. *16*, 431–444.
12. Liu, X., Jiang, N., Hughes, B., Bigras, E., Shoubridge, E., and Hekimi, S. (2005). Evolutionary conservation of the clk-1-dependent mechanism of longevity: loss of mclk1 increases cellular fitness and lifespan in mice. Genes Dev. *19*, 2424–2434.
13. Houtkooper, R.H., Mouchiroud, L., Ryu, D., Moullan, N., Katsyuba, E., Knott, G., Williams, R.W., and Auwerx, J. (2013). Mitonuclear protein imbalance as a conserved longevity mechanism. Nature *497*, 451–457.
14. Lee, S.J., Hwang, A.B., and Kenyon, C. (2010). Inhibition of respiration extends C. elegans life span via reactive oxygen species that increase HIF-1 activity. Curr. Biol. *20*, 2131–2136.
15. Khan, M.H., Ligon, M., Hussey, L.R., Hufnal, B., Farber, R., 2nd, Munkacsy, E., Rodriguez, A., Dillow, A., Kahlig, E., and Rea, S.L. (2013). TAF-4 is required for the life extension of isp-1, clk-1 and tpk-1 Mit mutants. Aging *5*, 741–758.
16. Hampton, M.B., Fadeel, B., and Orrenius, S. (1998). Redox regulation of the caspases during apoptosis. Ann. NY Acad. Sci. *854*, 328–335.
17. Zech, B., Kohl, R., von Knethen, A., and Brune, B. (2003). Nitric oxide donors inhibit formation of the Apaf-1/caspase-9 apoptosome and activation of caspases. Biochem. J. *371*, 1055–1064.
18. Butler, J.A., Mishur, R.J., Bhaskaran, S., and Rea, S.L. (2013). A metabolic signature for long life in the Caenorhabditis elegans Mit mutants. Aging Cell *12*, 130–138.
19. Judy, M.E., Nakamura, A., Huang, A., Grant, H., McCurdy, H., Weiberth, K.F., Gao, F., Coppola, G., Kenyon, C., and Kao, A.W. (2013). A shift to organismal stress resistance in programmed cell death mutants. PLoS Genet. *9*, e1003714.
20. Yeretssian, G., Correa, R.G., Doiron, K., Fitzgerald, P., Dillon, C.P., Green, D.R., Reed, J.C., and Saleh, M. (2011). Non-apoptotic role of BID in inflammation and innate immunity. Nature *474*, 96–99.

Center for Molecular Medicine, National Heart, Lung and Blood Institute, NIH Bethesda, MD 20892, USA.
*E-mail: finkelt@nih.gov

http://dx.doi.org/10.1016/j.cub.2014.05.057

Neurobiology: The Eye within the Brain

All vertebrates except mammals have photoreceptors within their brains; however, the light-sensitive cells have never been unambiguously identified. A new paper provides direct evidence of photosensitivity in cerebrospinal fluid (CSF)-contacting neurons in quail brain that mediate the seasonal reproductive response.

Michael Menaker

It is not difficult to make a cell — especially a nerve cell — responsive to visible light. In principle one has only to couple a light-sensitive molecule to an ion channel; the machinery to translate a change in membrane potential into action potentials is already in place. Natural selection has accomplished this many times in the long history of life; our recent ability to imitate this feat, more or less at will, has created the exciting new field of optogenetics. So it is somewhat surprising that biologists have found it hard to accept the evidence of direct photoreception by the vertebrate brain. More than 100 years ago the young Karl von Frisch — later a Nobel laureate and famed translator of the language of bees — and Ernest Scharrer trained blinded fish to come to the surface of the water for food in response to presentation of a light signal [1,2]. This led to the discovery of photosensitivity in the pineal gland and associated structures in fish and later in amphibians and reptiles [3]. Nonetheless it was with great skepticism that reproductive endocrinologists greeted the work of Benoit in the 1930s which showed (in retrospect quite clearly) that blinded ducks, like their sighted brothers, could be induced to grow their gonads by exposure to long days [4,5]. Benoit's work, which implicated hypothalamic photoreception, was viewed as a kind of curiosity and did not receive the further experimental attention that it deserved. In the 1960s we published several papers demonstrating that surgically blinded house sparrows synchronized (entrained) their circadian activity rhythms to light cycles and also responded reproductively to long days [6,7]. Work from several laboratories soon established that extra retinal or non-visual photoreception was an invariant feature of the sensory armametaria of all vertebrate classes, with the interesting and important exception of mammals [8]. The parallel story in mammals, involving specialized non-visual retinal photoreceptors — neither rods nor cones — is also fascinating [9,10]. A search for the photoreceptors mediating these responses in birds and reptiles was undertaken by several groups using two techniques: attempts to identify opsins (a class of protein usually associated with photoreceptors) in the brain with immunocytochemistry and the ingenious use of small radioluminous discs implanted in various regions of the brain to cause gonad growth [11–13]. These experiments identified brain regions that were putative sites of photoreception (Figure 1) and suggested cerebrospinal fluid (CSF)- contacting neurons as possible photoreceptive cell types [11,14].

It is likely that the skepticism with which discoveries in this field have been met is due in part to our own intensely visual sensory system and in part to the fact that no specific photoreceptors in the deep brain have been unambiguously identified. A new study by Yoshimura and colleagues [15] reported in this issue of *Current Biology* provides direct, neurophysiological evidence of photoreception by CSF-contacting neurons in the brains of quail linked to that bird's seasonal photoperiodic response.

The gold standard for the demonstration of intrinsic photosensitivity is neurophysiological recording of light responses from individual cells isolated from other possible photoreceptor inputs. This can be technically demanding especially if the putative photoreceptors are small cells buried in the deep brain. The authors have solved these technical problems elegantly. First they hypothesized that the CSF-contacting neurons in the paraventricular organ (PVO) were intrinsically photoreceptive. They had previously shown that these cells stained immunocytochemically with antibodies against OPN5 (an opsin

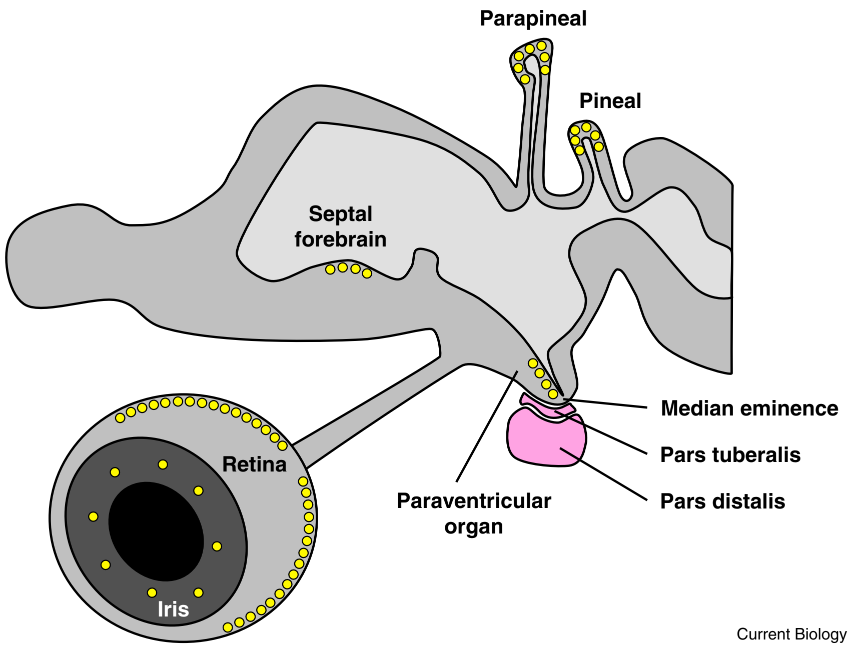

Figure 1. Non-visual photoreceptors in the vertebrate brain.

The parapineal and similar pineal-associated structures are only found in some fish, amphibians and reptiles, although the pineal itself is photoreceptive in all non-mammalian vertebrates. The iris is intrinsically photoreceptive in these groups as well and perhaps in some mammals. The putative locations of non-visual photoreceptors (shown in yellow) in the deep brain varies among the non-mammalian vertebrates. The adult mammalian pineal is not photoreceptive although it contains opsin. The only non-visual photoreceptors in mammals are intrinsically photosensitive ganglion cells in the retina. (Figure courtesy of I. Provencio.)

found in other non-visual structures) [16]. They then made slices containing the PVO from the brains of newly hatched quail, visualized the CSF-contacting neurons and recorded from them using whole cell patch clamp. They obtained very clear membrane depolarization and fast action potentials in response to light in this *in vitro* preparation. The depolarization response persisted when the action potentials were blocked by adding tetrodotoxin to the medium. Furthermore, the entire response to light including the fast action potentials was essentially unaffected when synaptic inputs to the cells were blocked with a cocktail containing antagonists to major neurotransmitters. This step demonstrated that the photosensitivity was indeed intrinsic to the cells from which the recordings were made and was not arriving as information from other photoreceptive cells via synapses. Finally they labeled the recorded cells with biocytin and stained them with anti-OPN5 antibody, confirming that the CSF-contacting neurons from which the recordings had been made did indeed contain OPN5. The results of these experiments provide clear proof that the CSF-contacting neurons are intrinsically photosensitive.

In a separate experiment using adult quail, they tested whether OPN5-mediated brain photoreception was involved in the seasonal reproductive response to long days. Quail were kept on short days, their pineal glands were surgically removed and their eyes covered leaving the deep brain as the only photoreceptive area potentially affected by light. They divided the birds into two groups and injected OPN5 siRNA into the third ventricle of the brain of one group (experimental) and scrambled OPN5 siRNA into the third ventricle of the other group (controls). One day later both groups were exposed to a long day and brains were collected at its end. The experimental group but not the controls showed two responses to the third ventricle injection: the number of CSF-contacting neurons staining with anti-OPN5 antibody was significantly reduced and the level of TSHB mRNA was reduced as well. Thyroid stimulating hormone (TSH) is normally induced by long days and is both an early indicator and an important regulator of the seasonal reproductive response [17].

Interpretation of the results of this experiment is not straightfoward. Injection of siRNA into the third ventricle is likely to affect all the CSF-contacting neurons and there may be others in locations outside the PVO that contain OPN5. Thus, we cannot be sure that CSF-contacting neurons in the PVO — which have been so clearly shown to be photoreceptive — are those that mediate the response of TSHB to the long day. So a firm connection between the photoreceptive OPN5-containing CSF-contacting neurons in the PVO and the reproductive response to season, which is all important in the lives of many birds, has yet to be made.

Working out the details of the photoreceptive response to long days is likely to be complicated. It will be technically difficult to determine the relative roles of other photoreceptive structures, of which there are several (Figure 1). Many different opsins have been found in the retina, pineal gland and the deep brain [18]. Non-visual photoreception is involved in several other partially overlapping functions: circadian entrainment, pupillary constriction, even (in mammals) fear conditioning [19]. Indeed, it is so widely present among the vertebrates that it constitutes a separate sensory modality distinct from vision. The current paper, although it does not provide final answers to the many questions raised by this complexity, is an elegant beginning to their in-depth analysis.

References

1. von Frish, K. (1911). Beiträge zur Physiologie der Pigmentzellen in der Fischhaut. Pflüger's Arch. *138*, 319–387.
2. Scharrer, E. (1928). Die Lichtempfindlichkeit blinder Elritzen. I Untersuchungen über das Zwischenhirn der Fische. Z. Vergl. Physiol. *7*, 1–38.
3. Dodt, E. (1973). The parietal eye (pineal and parietal organs of lower vertebrates). In Handbook of Sensory Physiology, Vol. VII/3B, Autrum, H., Jung, R., Lowenstein, W.R., Mckay, D.M., and Tueber, H.L., eds. (Berlin, New York: Springer-Verlag), pp. 113–140.
4. Benoit, J. (1935a). Le role des yeux dans l'action stimulante de la lumière sur le développement testiculaire chez le canard. C. R. Soc. Biol. *118*, 669–671.

5. Benoit, J. (1935b). Stimulation par la lumière artificielle du développement testiculair chez des canards aveugles par section du nerf optique. C. R. Soc. Biol. *120*, 133–136.
6. Menaker, M. (1968). Extraretinal light perception in the sparrow I: Entrainment of the biological clock. Proc. Natl. Acad. Sci. USA *59*, 414–421.
7. Menaker, M., and Keatts, H. (1968). Extraretinal light perception in the sparrow II: Photoperiodic stimulation of testis growth. Proc. Natl. Acad. Sci. USA *60*, 146–151.
8. Gerkema, M.P., Davies, W.I., Foster, R.G., Menaker, M., and Hut, R.A. (2013). The nocturnal bottleneck and the evolution of activity patterns in mammals. Proc. R Soc. B *280*, 21030508.
9. Provencio, I., Jiang, G., De Grip, W.J., Hayes, W.P., and Rollag, M.D. (1998). Melanopsin: an opsin in melanophores, brain, and eye. Proc. Natl. Acad. Sci. USA *95*, 340–345.
10. Berson, D.M., Dunn, F.A., and Takao, M. (2002). Phototransduction by retinal ganglion cells that set the circadian clock. Science *295*, 1070–1073.
11. Grace, M.S., Alones, V., Menaker, M., and Foster, R.G. (1996). Light perception in the vertebrate brain: an ultrastructural analysis of opsin – and vasoactive intestinal polypeptide-immunoreactive neurons in iguanid lizards. J. Comp. Neurol. *367*, 575–594.
12. Oliver, J., Jallageas, M., and Baylé, J.D. (1979). Plasma testerone and LH levels in male quail bearing hypothalamic lesions or radioluminous implants. Neuroendocrinology *28*, 114–122.
13. Homma, K., and Sakakibara, Y. (1971). Encephalic photoreceptors and their significance in photoperiodic control of sexual activity in Japanese quail. In Biochronometry, M. Menaker, ed. (Washington, D.C.: Natl. Acad. Sci), pp. 333–341.
14. Vigh, B., and Vigh-Teichmann, I. (1988). Comparative neurobiology and immunocytochemistry of the pineal complex with special reference to CSF-contacting neuronal structures. Pineal Res. Rev. *6*, 1–65.
15. Nakane, Y., Shimmura, T., Abe, H., and Yoshimura, T. (2014). Intrinsic photosensitivity of a deep brain photoreceptor. Curr. Biol. *24*, R596–R597.
16. Nakane, Y., Ikegami, K., Ono, H., Yamamoto, N., Yoshida, S., Hirunagi, K., Ebihara, S., Kubo, Y., and Yosimura, T. (2010). A novel mammalian neural tissue opsin (Opsin5) is a deep brain photoreceptor in birds. Proc. Natl. Acad. Sci. USA *107*, 15264–15268.
17. Nakao, N., Ono, H., Yamamura, T., Anraku, T., Takagi, T., Higashi, K., Yasuo, S., Katou, Y., Kageyama, S., Uno, Y., *et al*. (2008). Thyrotrophin in the pars tuberalis triggers photoperiodic response. Nature *452*, 317–322.
18. Peirson, S.N., Halford, S., and Foster, R.G. (2009). The evolution of irradiance detection: melanopsin and the non-visual opsins. Phil. Trans. R. Soc. B *364*, 2849–2865.
19. LeGates, T.A., Altimus, C.M., Wang, H., Lee, H.K., Yang, S., Zhao, H., Kirkwood, A., Weber, E.T., and Hattar, S. (2012). Aberrant light directly impairs mood and learning through melanopsin-expressing neurons. Nature *491*, 594–598.

Department of Biology, University of Virginia, Charlottesville, VA 22904-4328, USA.
E-mail: mm7e@virginia.edu

http://dx.doi.org/10.1016/j.cub.2014.05.037

Palaeontology: Which Came First, the Pterosaur or the Egg?

Pterosaur fossils are among the rarest and their eggs are even rarer. How then can we get an insight into the lifestyle of these flying reptiles? A wealth of new pterosaur fossils, including eggs, from China now provides exactly that.

David M. Martill

Pterosaurs are among the most awe inspiring of the archosaurian reptiles, vying with *Tyrannosaurus rex* and *Velociraptor* for a place at the top of the prehistoric popularity chart. The first pterosaurs described in the late 18th and early 19th centuries [1,2] were compared to the fiend of Dante's *Inferno* [3] despite their rather small size. Later discoveries, however, hinted at wingspans of more than 6.5 meters [4], and pterodactyls, more properly called pterosaurs, quickly became the dragons of popular folklore, and with star performances in Arthur Conan Doyle's *The Lost World* and Michael Crichton's *Jurassic Park*. Knowledge of the palaeoecology of pterosaurs has advanced at a painstakingly slow pace and their evolutionary relationships with other archosaurs, as well as within the Pterosauria, remain controversial [5]. Biomechanical studies of their flight had begun very early based on mathematic modelling of some extremely well preserved examples from the chalk of Kansas [6], but analysis of other aspects of their lifestyle, such as feeding and reproductive strategies, remain in their infancy even after more than 200 years of study. Now, in a recent issue of *Current Biology*, Wang *et al.* [7] report an extraordinary discovery of multiple partially articulated skeletons of a new genus and species of pterosaur named *Hamipterus tianshanensis*, associated with three-dimensionally preserved eggs.

Hardly any cases have been reported of well-preserved pterosaurs occurring as more than a single skeleton. Sure, there are a few bone beds with concentrations of pterosaur bones, most notably the Cretaceous Cambridge Greensand of England, but these deposits are a chaotic mix of very worn and broken fragments of at least five genera and it's the Devil's own job to sort out which bone belongs to which genus [8]. An exciting aspect of the discovery reported by Wang *et al.* [7] is that several exceptionally well preserved pterosaurs pertaining to a single taxon are associated, appearing to represent mature sub-adult and adult individuals. And what's more, at least five extremely well preserved eggs are mixed in with the bones.

Eggs First

Until ten years ago, the only evidence that pterosaurs laid eggs with mineralised shells — as opposed to soft-shelled eggs or giving birth to live young — came from phylogenetic analyses that showed pterosaurs as a sister taxon to Dinosauria forming the clade Ornithodira (but usually called Avemetatarsalia [9]). This clade comprising dinosaurs, birds and pterosaurs is in turn a sister clade to one that includes extant crocodiles. Thus, in the great tree of life, pterosaurs lie between crocodiles and birds, both of which lay eggs with a calcite shell. Parsimony thus suggests that pterosaurs too laid eggs with a calcite shell. Known as extant phylogenetic bracketing, the assumption is that birds and crocodiles inherited calcite shelled eggs from a shared common ancestor and pterosaurs should also have done so. However, it is possible that the pterosaur clade might at some time in their early evolution have lost the shelled egg, perhaps laying soft eggs, or perhaps not even laying eggs at all. The fossil record might just throw up a soft-shelled egg, but live birth would require the death and preservation of a gravid female, known for ichthyosaurs, but not for pterosaurs.

The first discovery of a fossil pterosaur egg came from the Early