SUBMITTED VERSION

This is the pre-peer reviewed version of the following article:

Daniel S Narayan, Glyn Chidlow, John PM Wood and Robert J Casson
Glucose metabolism in mammalian photoreceptor inner and outer segments
Clinical and Experimental Ophthalmology, 2017; 45(7):730-741

which has been published in final form at http://dx.doi.org/10.1111/ceo.12952.
This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Use of Self-Archived Versions."

http://hdl.handle.net/2440/129408

Glucose Metabolism in Mammalian Cones

[1,2]Daniel S. Narayan MBBS, [1,2]Glyn Chidlow DPhil, [1,2]John P.M. Wood DPhil, [1,2]Robert J. Casson DPhil, FRANZCO

[1]*Ophthalmic Research Laboratories, Hanson Institute Centre for Neurological Diseases, Frome Rd, Adelaide, SA 5000, Australia*

[2]*South Australian Institute of Ophthalmology, University of Adelaide, Frome Rd, Adelaide, SA 5000, Australia*

Address correspondence to:

Daniel S. Narayan

Department of Ophthalmology, Royal Adelaide Hospital, North Tce., Adelaide, South Australia, Australia, 5000.

Email: daniel.narayan@adelaide.edu.au

Tel:+61 8 8222 2729

Fax:+61 8 8222 3392

Running Title: Glucose Metabolism in Cones

Conflict of Interest Disclosures: None.

Introduction

Photoreceptors are the first order neurons of the visual pathway, converting light into electrical signals. There are three known types of photoreceptors in the mammalian retina: rods and cones (the classical photoreceptors) and photosensitive retinal ganglion cells (pRGCs). Rods have low spatial resolution but are extremely sensitive to light. They are specialized for sensitivity at the expense of resolution and are responsible for vision in dimly lit conditions. Cones have high spatial resolution but are relatively insensitive to light and are responsible for high acuity central vision and colour vision. pRGCs mainly have subcortical targets, with minimal visual processing and are not a subject for this review. (For the remainder of this review, when we refer to "photoreceptors" we mean the classical photoreceptors.) Many diseases of the retina involve degeneration of photoreceptors. These include inherited diseases such as retinitis pigmentosa, as well as acquired diseases such as diabetic retinopathy, age-related macular degeneration, central retinal artery occlusion and central retinal vein occlusion. These diseases currently have limited treatment options and are responsible for a large portion of visual impairment worldwide. Because loss of central vision is so debilitating to quality of life in humans [1], preservation of cone function in retinal disease is a major therapeutic goal. Photoreceptors are highly metabolically active cells and account for much of the energy consumption in the retina [2-4]. The major photoreceptor energy substrate *in vivo* is glucose [5-7]. Energy failure, which refers to the relative deficiency of substrates required to make energy for cellular processes, has been suggested to play a role in a number of diseases of photoreceptor degeneration [8, 9]. Recent studies suggest that bio-energetic therapies may help preserve cones in many models of photoreceptor degeneration [9-11]. However, the pathways by which cones meet their energy demands remain incompletely understood. Improvements in the

understanding of glucose metabolism in cones may provide insight into the reasons why cones degenerate due to energy failure. This may, in turn, assist in developing bio-energetic therapies aimed at protecting cones.

Structure and Functions of Cones

The mammalian retina, like all vertebrate retinas, comprises two synaptic layers intercalated between three neuronal body layers[12]. In vertebrates, photoreceptors are situated in the outer retina. In the human retina, rods account for approximately 95% of photoreceptors and cones account for the remainder. The density of rods and cones varies with retinal eccentricity: rods dominate the peripheral retina in humans; cone density increases towards the macula and the foveola is exclusively cones.

Photoreceptors are long narrow cells that consist of four primary structural and functional regions: outer segments, inner segments, cell bodies, and synaptic terminals (Fig. 1). The inner and outer segments are connected by a stalk of modified cilium and separated from the cell body by the outer limiting membrane. Photoreceptor cell nuclei are situated in the outer nuclear layer of the retina and the axons pass into the outer plexiform layer where they form synaptic terminals with bipolar cells and horizontal cells. Photoreceptor outer segments contain the visual pigments, called opsins, responsible for absorption of light and initiation of neuro-electrical impulse.

The different architectures of rod and cone outer segments represent a major distinctive feature of the two photoreceptor cell types. Cones are conical shaped cells and their outer segments are generally shorter than rods. Like rods, the visual pigment proteins in cones are arranged as discs in the outer segments. Unlike rods, however, the discs in cones are not surrounded by a plasma membrane [13-15]. Instead, they are in free

communication with the intracellular space. In addition, rods and cones possess different visual pigment proteins (opsins). Rhodopsin, which is a G-protein receptor, is the primary light-sensitive visual protein found in rods. Cone opsins, however, can be divided into a number of subgroups, which correspond to their absorption spectra: long wavelength opsins, middle wavelength opsins and short wavelength opsins.

The human retina receives blood supply from two different vascular beds. The inner two-thirds of the retina are nourished by branches from the central retinal vessels. The outer third of the retina, including the photoreceptors, is nourished by the choroidal circulation. Blood from the choroidal vessels traverses an outer and an inner blood-retinal barrier before reaching the photoreceptors. The outer barrier is the retinal pigment epithelium (RPE) interposed between the choriocapillaries and the avascular outer retina, and the inner barrier is composed of endothelial cells within retinal capillaries [16]. Both barriers are mediated by tight junctions, but both cell types have facilitated glucose transporters that permit passive movement of glucose across their plasma membranes [17, 18].

Glucose Delivery to Cones

Glucose is the preferred energy substrate for the retina and is converted intracellularly to adenosine triphosphate (ATP), which is used to transport chemical energy used for metabolism [5-7, 19]. When glucose is scarce, photoreceptors are known to have the capacity to take up and metabolize lactate [20, 21]. A potential source of lactate is the neighboring Müller glial cell. However, there is controversy as to whether photoreceptors metabolize significant amounts of lactate under normal physiological conditions [22-24]. Another source of energy for photoreceptors is provided by creatine

kinase (CK), which transfers high-energy phosphate groups from creatine phosphate to ATP. CK is present in photoreceptor outer segments and it has been suggested that a phosphocreatine shuttle pathway transports high-energy phosphate groups from the inner segment to the outer segment [2, 25-28].

The delivery of glucose to photoreceptors is crucially dependent on Glut1, which is the most widely expressed member of the Glut family of facilitative glucose transporters[17, 29-33]. Glut1 transports glucose from the choroidal vasculature to the outer retina across the blood–retina barrier formed by the RPE. Both the apical and basolateral membranes of RPE cells contain Glut1 transporters allowing direct passage of glucose down its concentration gradient to the retina [34, 35]. Glut1 also mediates glucose uptake by the photoreceptors themselves, because it is the only known glucose transporter expressed by photoreceptors [17]. Glut1 function in cones is dependent on a trophic factor produced by rods. Rod-derived cone viability factor (RdCVF) is released by rods and interacts with a complex formed by basignin-1 and Glut1 on the cell surface of cones, which accelerates intracellular glucose uptake in cones [10]. Current evidence suggests that Glut1 receptors are concentrated in the photoreceptor inner segments, and are actively excluded in the outer segments [29].

Energy Consumption in Cones

The retina has one of the highest energy demands of any tissue in the human body [36-38]. Photoreceptors are the most metabolically active cells in the retina and account for the majority of retinal energy consumption [2-4]. Active transport of ions against their concentration and electrical gradients is the largest energy consuming function in all neurons, including photoreceptors [3, 39-41]. Energy consumption within photoreceptors is

compartmentalized and light-dependent (Fig. 2). During illumination, photo-transduction and light adaptation consume energy in the outer segment. In darkness, energy is consumed by ion pumps in the inner segment and by glutamate release at the synaptic terminal. The remainder of energy expenditure is used for the synthesis, recycling and transport of molecules such as opsins and neurotransmitters [3]. Energy demands and oxygen consumption in photoreceptors is more than three-fold greater in darkness than in light [2, 38].

In the dark, a steady current flows into open channels in the photoreceptor cell membrane, which partially depolarizes the photoreceptor cell. The depolarized photoreceptor releases glutamate from its synaptic terminals upon second-order neurons in the dark. This "dark current" is composed mainly of the influx of sodium and calcium ions. In darkness, excess sodium and calcium ions are removed via Na+K+ATPase pumps and Ca++ATPase pumps. These pumps accounts for more than 50% of the energy consumption of photoreceptors in darkness and 15% in light [38]. The ion pumps that maintain the dark current are heavily concentrated in the inner segments, which also house the majority of the mitochondria and Glut1 receptors. The amplitude and voltage-dependence of the dark current is similar in rods and cones and, therefore, both types of photoreceptors expend similar amounts of energy in darkness [42-44].

The second largest energy expending process in photoreceptors is photo-transduction. The process of photo-transduction occurs in a stepwise process shared by rods and cones [15]. Light induces the isomerisation of the chromophore 11-cis-retinal to all-trans-retinal, which dissociates from opsin and is reduced by retinol dehydrogenase and its cofactor nicotinamide adenine dinucleotide phosphate (NADPH) to all-trans-

retinol. The rate of this reduction is 10 to 40 times higher in cones than in rods. All-trans-retinol is transported to the RPE, where it is isomerised and oxidized back to 11-cis-retinal, which is recycled to photoreceptor cells. Meanwhile, opsin undergoes a conformational change and activates the G-protein transducin. This replaces guanosine diphosphate (GDP) with guanosine triphosphate (GTP) and activates the α-subunit of transducin which then dissociates and activates phosphodiesterase (PDE) by removing two regulatory (γ) subunits. The activated PDE then hydrolyses cyclic guanosine monophosphate (cGMP) to 5'-GMP. A decrease in cGMP concentration leads to the closing of ion channels in the outer segments and photoreceptor cells hyperpolarize to light. Afterwards, GTP is hydrolysed back to GDP in preparation for the next cycle, and opsin is phosphorylated by opsin kinase to interact with arrestin for its own inactivation.

All of these processes involve GTP or ATP. The total amount of ATP consumed varies among species and depends upon the intensity and duration of light exposure. However, all of the energy expenditure of photo-transduction is still relatively small compared to that of the dark current [2, 41]. The energy consuming processes in photo-transduction occur primarily in the outer segments where the opsin proteins are located. This area is devoid of mitochondria and lacks Glut1 receptors.

Although rods and cones consume similar amounts of energy in darkness, cones consume more overall energy than rods because they do not saturate in bright light and they use more energy for photo-transduction [2, 45-48]. In bright light, sodium ion influx through cGMP-gated channels in cones does not fall below half that in darkness, the turnover number of transducin is at least twice as that in rods [47], opsin kinase activity

is also much higher in cones [48], and cones depolarize more frequently than rods. The higher energy requirements of cones are also facilitated by a greater quantity of oxidative machinery; cones posses twice as many mitochondria as rods [2, 49].

Glucose Metabolism Pathways in Cones

The metabolism of glucose in cones supports several key activities that are essential for cone survival. ATP production is an important outcome of glucose metabolism. Intracellular ATP is generated via two related metabolic pathways: oxidative phosphorylation and glycolysis [50]. The energetic advantage of oxidative phosphorylation in the mitochondria far outweighs that of glycolysis in the cytosol, as one molecule of glucose yields only two net molecules of ATP via the glycolytic pathway but 34 net molecules of ATP via the oxidative pathway. In most cells, pyruvate, which is made from glucose via glycolysis, is converted to lactate when oxygen is scarce. However, cultured retinal explants produce large amounts of lactate even under aerobic conditions [51]. Also, increasing oxygen delivery in the retina does not affect glucose consumption or lactate production, which suggests the retina relies on aerobic glycolysis [38, 52].

Oxygen consumption generally parallels ATP consumption in photoreceptors. Like ATP consumption, photoreceptor oxygen consumption is greater in the dark than in light [53, 54]. However, the rate of oxygen consumption in inner segments is five-fold greater than in outer segments, which is disproportionate to the rate of energy consumption [36, 55]. This suggests photoreceptors outer segments utilise ATP produced from oxidative phosphorylation in the inner segment and via glycolysis in the outer segment.

The inner retina metabolises 21% of glucose via glycolysis and 69% via oxidative phosphorylation [56, 57]. In contrast, the outer retina metabolises 61% of glucose via glycolysis and 12% via oxidative phosphorylation [56, 57]. Despite the high rates of glycolysis, ATP production from glycolysis constitutes only 16% of available ATP in photoreceptors in both darkness and light [3, 38]. ATP production from oxidative phosphorylation constitutes 84% of available ATP in darkness and 61% in the light [3, 38].

Photoreceptors are responsible for the majority of glycolysis in the retina. This is supported by the fact that photoreceptors are particularly susceptible to glycolytic inhibition, and, in rat retinas that lack photoreceptor cells, there is greater than 50% reduction in the overall glycolytic activity compared to the normal rat retina [58]. Additionally, expression of lactate dehydrogenase-A (LDH-A), which catalyses the conversion of pyruvate to lactate and serves as a biomarker for glycolysis, is also reduced in rat retinas that lack photoreceptors [59].

Photoreceptors are highly polarized cells that sequester key biochemical reactions in anatomically distinct compartments [2]. For example, the ion pumps responsible for maintaining the dark current are localized to the photoreceptor inner segment, where numerous mitochondria supply ATP through oxidative phosphorylation [60]. This is supported by the finding that concentrations of key oxidative enzymes, such as malate dehydrogenase in monkeys, is up to 30 times higher in inner segments than in outer segments [61].

Conversely, the energy consuming reactions that occur in light take place in the photoreceptor outer segment. Because the outer segment is devoid of mitochondria, it has been proposed that the energy used in this compartment might be supplied from the inner segment by diffusion of ATP through the connecting cilium [26]. However, the presence of glycolytic enzymes in the outer segment, as determined by biochemical and immunohistochemical methods, suggests that the outer segment is not entirely dependent on inner segment metabolism [27, 30, 62, 63]. Rather, it appears to possess the energy-generating capacity to meet at least some of its own needs through glycolysis [29]. ATP supply is also supported by a creatine shuttle transporting ATP, produced via oxidative phosphorylation, from the inner segment to the outer segment [25, 27, 64]. In addition, the presence of ectopic mitochondrial proteins in the outer segment suggests that some ATP may also be produced via oxidative phosphorylation in the outer segment [65, 66].

The reliance of photoreceptors on aerobic glycolysis is highly unusual. The metabolism of photoreceptors more closely resembles that of rapidly proliferating or dividing cells instead of other neurons [67]. Since photoreceptors have high-energy demands, it is unclear why they metabolise the majority of available glucose using glycolysis, which produces less net energy than oxidative phosphorylation. One possible explanation for this phenomenon is that, apart from using glucose for energy production, photoreceptors may also use glucose to produce metabolic intermediates for amino acid biosynthesis [68]. Rapidly proliferating neoplastic cells also use this strategy. Provided that the energy supply is sufficient, glycolytic metabolites become diverted towards biosynthesis rather than towards oxidative phosphorylation [69]. The molecular switch controlling glycolytic flow is thought to be an isoenzyme of pyruvate

kinase (PKM2), which is specifically localized to photoreceptors in the mammalian retina [70].

Adult mammalian photoreceptors are non-proliferative, however, they posses high biosynthesis requirements due to the prodigious turnover of the opsin proteins in the disc membranes of the outer segments [67, 71, 72]. Both rod and cone outer segments are renewed in an orderly fashion, as first revealed by autoradiographic studies in which radioactive amino acids became trapped in new membranous discs generated at the outer segment base, moved towards the photoreceptor tip, and were finally phagocytosed by the RPE. [71, 72].

The visual pigment proteins in photoreceptor outer segments share several common amino acid motifs. Their distinct molecular properties arise from differences in the residues at positions 122 and 189 of the amino-acid sequence [73-75]. Many of the amino acids used to form opsin molecules, such as serine and glycine, can be produced through glycolysis. Hence, glycolytic intermediates have the potential to be incorporated into the production of opsin proteins.

Glucose metabolism in cones serves a number of other functions in addition to ATP production and opsin synthesis. Glucose contributes to synthesis of N-acetylglucosamine, which is required for asparagine N-linked protein glycosylation and subsequent trafficking to the cell surface [76, 77]. Proper trafficking of transporters and growth factor receptors is required for glucose uptake and cellular responses to pro-survival signals [77, 78]. Glucose is also needed for production of cytosolic NADPH, which can inhibit caspase-mediated apoptosis, support anabolic activity, and help to maintain

appropriate levels of reactive oxygen species [79-83]. There is also evidence that glucose is directed into the pentose phosphate pathway in photoreceptor outer segments which generates nucleotides and NADPH, and possibly also makes the amino acids, serine and glycine, branching from glycolysis at phosphoglycerate [69, 84, 85].

Clinical Implications

Degeneration of photoreceptors causes blindness in a variety of retinal diseases including retinitis pigmentosa, diabetic retinopathy and age-related macular degeneration [86-88]. In diseases of photoreceptor degeneration, the main cause of clinically significant vision loss is cone cell degeneration rather than rod cell death [1]. Thus, prevention of cone cell loss is a major goal of therapeutic strategies [89]. The pathways that lead to photoreceptor degeneration are diverse and incompletely understood.

Cones consume large amounts of energy and impose a higher metabolic cost than rods. Therefore, energy metabolism may be a critical juncture that links cone function and survival. Retinitis pigmentosa is an example of a condition in which cone survival may be critically related to energy supply [9-11]. Although, most genetic mutations responsible for retinitis pigmentosa in humans and animal models affect rod-specific genes, rod degeneration is often followed by secondary cone degeneration. New evidence suggests rod degeneration results in reduced levels of RdCVF, which can lead to cone degeneration via a mechanism that involves energy failure [10]. Furthermore, subretinal RdCVF administration decreases the rate of cone loss by increasing glucose uptake in retinal degeneration mice [10, 90]. In addition, activation of the mechanistic target of rapamycin (mTOR) signalling pathway increases cone survival in retinal

degeneration mice partly by increasing the availability of energy substrates [9, 11].

Lack of suitable experimental mammalian models constitutes a major barrier to improving our understanding of cone pathophysiology. Due to the rarity of cones and the fragility of cone outer segments, it is difficult to produce purified mammalian cone cell cultures for in vitro studies [91]. Also, rats and mice are primarily used for in vivo studies of photoreceptor degeneration. However, they have a dearth of cones and are of limited use for cone research [92]. Animals with cone dominance, such as ground squirrels (85% cones) [93-95], chickens (65% cones) [96], and pigs (20% cones) [97] are difficult to breed in captivity. However, emerging models of cone-rich mouse and rat strains may provide future opportunities for in vivo study [98-101]. Ultimately, improving our understanding of cone structure and function will assist in developing therapies that protect cones.

References

1. Mustafi D, Engel AH, Palczewski K. Structure of cone photoreceptors. Prog Retin Eye Res 2009; **28:** 289-302.

2. Okawa H, Sampath AP, Laughlin SB, Fain GL. ATP consumption by mammalian rod photoreceptors in darkness and in light. Curr Biol 2008; **18:** 1917-21.

3. Wong-Riley MT. Energy metabolism of the visual system. Eye Brain 2010; **2:** 99-116.

4. Wong-Riley MT, Huang Z, Liebl W, Nie F, Xu H, Zhang C. Neurochemical organization of the macaque retina: effect of TTX on levels and gene expression of cytochrome oxidase and nitric oxide synthase and on the immunoreactivity of Na+ K+ ATPase and NMDA receptor subunit I. Vision Res 1998; **38:** 1455-77.

5. Agathocleous M, Love NK, Randlett O, Harris JJ, Liu J, Murray AJ *et al.* Metabolic differentiation in the embryonic retina. Nat Cell Biol 2012; **14:** 859-64.

6. Cohen LH, Noell WK. Glucose catabolism of rabbit retina before and after development of visual function. J Neurochem 1960; **5:** 253-76.

7. Winkler BS, Pourcho RG, Starnes C, Slocum J, Slocum N. Metabolic mapping in mammalian retina: a biochemical and 3H-2-deoxyglucose autoradiographic study. Exp Eye Res 2003; **77:** 327-37.

8. Feigl B. Age-related maculopathy - linking aetiology and pathophysiological changes to the ischaemia hypothesis. Prog Retin Eye Res 2009; **28:** 63-86.

9. Punzo C, Kornacker K, Cepko CL. Stimulation of the insulin/mTOR pathway delays cone death in a mouse model of retinitis pigmentosa. Nat Neurosci 2009; **12:** 44-52.

10. Ait-Ali N, Fridlich R, Millet-Puel G, Clerin E, Delalande F, Jaillard C *et al.* Rod-derived cone viability factor promotes cone survival by stimulating aerobic glycolysis. Cell 2015; **161:** 817-32.

11. Venkatesh A, Ma S, Le YZ, Hall MN, Ruegg MA, Punzo C. Activated mTORC1 promotes long-term cone survival in retinitis pigmentosa mice. J Clin Invest 2015; **125:** 1446-58.

12.

13. Nilsson SE. THE ULTRASTRUCTURE OF THE RECEPTOR OUTER SEGMENTS IN THE RETINA OF THE LEOPARD FROG (RANA PIPIENS). J Ultrastruct Res 1965; **12:** 207-31.

14. Cohen AI. New evidence supporting the linkage to extracellular space of outer segment saccules of frog cones but not rods. J Cell Biol 1968; **37:** 424-44.

15. Yau KW. Phototransduction mechanism in retinal rods and cones. The Friedenwald Lecture. Invest Ophthalmol Vis Sci 1994; **35:** 9-32.

16. Cunha-Vaz JG. The blood-retinal barriers. Doc Ophthalmol 1976; **41:** 287-327.

17. Mantych GJ, Hageman GS, Devaskar SU. Characterization of glucose transporter isoforms in the adult and developing human eye. Endocrinology 1993; **133:** 600-7.

18. Kumagai AK, Glasgow BJ, Pardridge WM. GLUT1 glucose transporter expression in the diabetic and nondiabetic human eye. Invest Ophthalmol Vis Sci 1994; **35:** 2887-94.

19. Wood JP, Chidlow G, Graham M, Osborne NN. Energy substrate requirements for survival of rat retinal cells in culture: the importance of glucose and monocarboxylates. J Neurochem 2005; **93:** 686-97.

20. Winkler BS. Glycolytic and oxidative metabolism in relation to retinal function. J Gen Physiol 1981; **77:** 667-92.

21. Poitry-Yamate CL, Poitry S, Tsacopoulos M. Lactate released by Muller glial cells is metabolized by photoreceptors from mammalian retina. J Neurosci 1995; **15:** 5179-91.

22. Chih CP, Lipton P, Roberts EL, Jr. Do active cerebral neurons really use lactate rather than glucose? Trends Neurosci 2001; **24:** 573-8.

23. Winkler BS, Starnes CA, Sauer MW, Firouzgan Z, Chen SC. Cultured retinal neuronal cells and Muller cells both show net production of lactate. Neurochem Int 2004; **45:** 311-20.

24. Acosta ML, Fletcher EL, Azizoglu S, Foster LE, Farber DB, Kalloniatis M. Early markers of retinal degeneration in rd/rd mice. Mol Vis 2005; **11:** 717-28.

25. Wallimann T, Wegmann G, Moser H, Huber R, Eppenberger HM. High content of creatine kinase in chicken retina: compartmentalized localization of creatine kinase isoenzymes in photoreceptor cells. Proc Natl Acad Sci U S A 1986; **83:** 3816-9.

26. Linton JD, Holzhausen LC, Babai N, Song H, Miyagishima KJ, Stearns GW *et al.* Flow of energy in the outer retina in darkness and in light. Proc Natl Acad Sci U S A 2010; **107:** 8599-604.

27. Hsu SC, Molday RS. Glucose metabolism in photoreceptor outer segments. Its role in phototransduction and in NADPH-requiring reactions. J Biol Chem 1994; **269:** 17954-9.

28. Tachikawa M, Hosoya K, Ohtsuki S, Terasaki T. A novel relationship between creatine transport at the blood-brain and blood-retinal barriers, creatine biosynthesis, and its use for brain and retinal energy homeostasis. Subcell Biochem 2007; **46:** 83-98.

29. Gospe SM, 3rd, Baker SA, Arshavsky VY. Facilitative glucose transporter Glut1 is actively excluded from rod outer segments. J Cell Sci 2010; **123:** 3639-44.

30. Hsu SC, Molday RS. Glycolytic enzymes and a GLUT-1 glucose transporter in the outer segments of rod and cone photoreceptor cells. J Biol Chem 1991; **266:** 21745-52.

31. Nihira M, Anderson K, Gorin FA, Burns MS. Primate rod and cone photoreceptors may differ in glucose accessibility. Invest Ophthalmol Vis Sci 1995; **36:** 1259-70.

32. Elliott MH, Nash ZA, Takemori N, Fliesler SJ, McClellan ME, Naash MI. Differential distribution of proteins and lipids in detergent-resistant and detergent-soluble domains in rod outer segment plasma membranes and disks. J Neurochem 2008; **104:** 336-52.

33. Lopez-Escalera R, Li XB, Szerencsei RT, Schnetkamp PP. Glycolysis and glucose uptake in intact outer segments isolated from bovine retinal rods. Biochemistry 1991; **30:** 8970-6.

34. Takata K, Kasahara T, Kasahara M, Ezaki O, Hirano H. Erythrocyte/HepG2-type glucose transporter is concentrated in cells of blood-tissue barriers. Biochem Biophys Res Commun 1990; **173:** 67-73.

35. Takata K, Kasahara T, Kasahara M, Ezaki O, Hirano H. Ultracytochemical localization of the erythrocyte/HepG2-type glucose transporter (GLUT1) in cells of the blood-retinal barrier in the rat. Invest Ophthalmol Vis Sci 1992; **33:** 377-83.

36. Yu DY, Cringle SJ. Oxygen distribution and consumption within the retina in vascularised and avascular retinas and in animal models of retinal disease. Prog Retin Eye Res 2001; **20:** 175-208.

37. Anderson B, Jr., Saltzman HA. RETINAL OXYGEN UTILIZATION MEASURED BY HYPERBARIC BLACKOUT. Arch Ophthalmol 1964; **72:** 792-5.

38. Ames A, 3rd, Li YY, Heher EC, Kimble CR. Energy metabolism of rabbit retina as related to function: high cost of Na+ transport. J Neurosci 1992; **12:** 840-53.

39. Niven JE, Laughlin SB. Energy limitation as a selective pressure on the evolution of sensory systems. J Exp Biol 2008; **211:** 1792-804.

40. Harris JJ, Attwell D. The energetics of CNS white matter. J Neurosci 2012; **32:** 356-71.

41. Wong-Riley MT. Cytochrome oxidase: an endogenous metabolic marker for neuronal activity. Trends Neurosci 1989; **12:** 94-101.

42. Yagi T, Macleish PR. Ionic conductances of monkey solitary cone inner segments. J Neurophysiol 1994; **71:** 656-65.

43. Nikonov SS, Kholodenko R, Lem J, Pugh EN, Jr. Physiological features of the S- and M-cone photoreceptors of wild-type mice from single-cell recordings. J Gen Physiol 2006; **127:** 359-74.

44. Thoreson WB, Tranchina D, Witkovsky P. Kinetics of synaptic transfer from rods and cones to horizontal cells in the salamander retina. Neuroscience 2003; **122:** 785-98.

45. Matthews HR, Fain GL, Murphy RL, Lamb TD. Light adaptation in cone photoreceptors of the salamander: a role for cytoplasmic calcium. J Physiol 1990; **420:** 447-69.

46. Burkhardt DA. Light adaptation and photopigment bleaching in cone photoreceptors in situ in the retina of the turtle. J Neurosci 1994; **14:** 1091-105.

47. Nikonov SS, Brown BM, Davis JA, Zuniga FI, Bragin A, Pugh EN, Jr. *et al.* Mouse cones require an arrestin for normal inactivation of phototransduction. Neuron 2008; **59:** 462-74.

48. Kawamura S, Tachibanaki S. Rod and cone photoreceptors: molecular basis of the difference in their physiology. Comp Biochem Physiol A Mol Integr Physiol 2008; **150:** 369-77.

49. Perkins GA, Ellisman MH, Fox DA. Three-dimensional analysis of mouse rod and cone mitochondrial cristae architecture: bioenergetic and functional implications. Mol Vis 2003; **9:** 60-73.

50. Romano AH, Conway T. Evolution of carbohydrate metabolic pathways. Res Microbiol 1996; **147:** 448-55.

51.

52. Wang L, Bill A. Effects of constant and flickering light on retinal metabolism in rabbits. Acta Ophthalmol Scand 1997; **75:** 227-31.

53. Lau JC, Linsenmeier RA. Oxygen consumption and distribution in the Long-Evans rat retina. Exp Eye Res 2012; **102:** 50-8.

54. Linsenmeier RA. Effects of light and darkness on oxygen distribution and consumption in the cat retina. J Gen Physiol 1986; **88:** 521-42.

55. Yu DY, Cringle SJ, Su EN. Intraretinal oxygen distribution in the monkey retina and the response to systemic hyperoxia. Invest Ophthalmol Vis Sci 2005; **46:** 4728-33.

56. Wang L, Tornquist P, Bill A. Glucose metabolism in pig outer retina in light and darkness. Acta Physiol Scand 1997; **160:** 75-81.

57. Wang L, Tornquist P, Bill A. Glucose metabolism of the inner retina in pigs in darkness and light. Acta Physiol Scand 1997; **160:** 71-4.

58. Noell WK. The effect of iodoacetate on the vertebrate retina. J Cell Physiol 1951; **37:** 283-307.

59. Graymore C. POSSIBLE SIGNIFICANCE OF THE ISOENZYMES OF LACTIC DEHYDROGENASE IN THE RETINA OF THE RAT. Nature 1964; **201:** 615-6.

60. Stahl WL, Baskin DG. Immunocytochemical localization of Na+,K+ adenosine triphosphatase in the rat retina. J Histochem Cytochem 1984; **32:** 248-50.

61. Lowry OH, Roberts NR, Lewis C. The quantitative histochemistry of the retina. J Biol Chem 1956; **220:** 879-92.

62. McConnell DG, Ozga GW, Solze DA. Evidence for glycolysis in bovine retinal microsomes and photoreceptor outer segments. Biochim Biophys Acta 1969; **184:** 11-28.

63. Lowry OH, Roberts NR, Schulz DW, Clow JE, Clark JR. Quantitative histochemistry of retina. II. Enzymes of glucose metabolism. J Biol Chem 1961; **236:** 2813-20.

64. Tombes RM, Shapiro BM. Metabolite channeling: a phosphorylcreatine shuttle to mediate high energy phosphate transport between sperm mitochondrion and tail. Cell 1985; **41:** 325-34.

65. Panfoli I, Calzia D, Ravera S, Bruschi M, Tacchetti C, Candiani S *et al.* Extramitochondrial tricarboxylic acid cycle in retinal rod outer segments. Biochimie 2011; **93:** 1565-75.

66. Panfoli I, Musante L, Bachi A, Ravera S, Calzia D, Cattaneo A *et al.* Proteomic analysis of the retinal rod outer segment disks. J Proteome Res 2008; **7:** 2654-69.

67. Ng SK, Wood JP, Chidlow G, Han G, Kittipassorn T, Peet DJ *et al.* Cancer-like metabolism of the mammalian retina. Clin Experiment Ophthalmol 2015; **43:** 367-76.

68. Casson RJ, Chidlow G, Han G, Wood JP. An explanation for the Warburg effect in the adult mammalian retina. Clin Experiment Ophthalmol 2013; **41:** 517.

69. Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. Science 2009; **324:** 1029-33.

70. R JC, J PMW, Han G, Kittipassorn T, D JP, Chidlow G. M-Type Pyruvate Kinase Isoforms and Lactate Dehydrogenase A in the Mammalian Retina: Metabolic Implications. Invest Ophthalmol Vis Sci 2016; **57:** 66-80.

71. Young RW. The renewal of photoreceptor cell outer segments. J Cell Biol 1967; **33:** 61-72.

72. Young RW. The renewal of rod and cone outer segments in the rhesus monkey. J Cell Biol 1971; **49:** 303-18.

73. Imai H, Kojima D, Oura T, Tachibanaki S, Terakita A, Shichida Y. Single amino acid residue as a functional determinant of rod and cone visual pigments. Proc Natl Acad Sci U S A 1997; **94:** 2322-6.

74. Kuwayama S, Imai H, Hirano T, Terakita A, Shichida Y. Conserved proline residue at position 189 in cone visual pigments as a determinant of molecular properties different from rhodopsins. Biochemistry 2002; **41:** 15245-52.

75. Shichida Y, Matsuyama T. Evolution of opsins and phototransduction. Philos Trans R Soc Lond B Biol Sci 2009; **364:** 2881-95.

76. Chertov AO, Holzhausen L, Kuok IT, Couron D, Parker E, Linton JD *et al.* Roles of glucose in photoreceptor survival. J Biol Chem 2011; **286:** 34700-11.

77. Asano T, Katagiri H, Takata K, Lin JL, Ishihara H, Inukai K *et al.* The role of N-glycosylation of GLUT1 for glucose transport activity. J Biol Chem 1991; **266:** 24632-6.

78. Wellen KE, Lu C, Mancuso A, Lemons JM, Ryczko M, Dennis JW *et al.* The hexosamine biosynthetic pathway couples growth factor-induced glutamine uptake to glucose metabolism. Genes Dev 2010; **24:** 2784-99.

79. Reidel B, Thompson JW, Farsiu S, Moseley MA, Skiba NP, Arshavsky VY. Proteomic profiling of a layered tissue reveals unique glycolytic specializations of photoreceptor cells. Mol Cell Proteomics 2011; **10:** M110.002469.

80. Vaughn AE, Deshmukh M. Glucose metabolism inhibits apoptosis in neurons and cancer cells by redox inactivation of cytochrome c. Nat Cell Biol 2008; **10:** 1477-83.

81. Yang CS, Thomenius MJ, Gan EC, Tang W, Freel CD, Merritt TJ *et al.* Metabolic regulation of Drosophila apoptosis through inhibitory phosphorylation of Dronc. Embo j 2010; **29:** 3196-207.

82. Futterman S, Hendrickson A, Bishop PE, Rollins MH, Vacano E. Metabolism of glucose and reduction of retinaldehyde in retinal photoreceptors. J Neurochem 1970; **17:** 149-56.

83. Groeger G, Mackey AM, Pettigrew CA, Bhatt L, Cotter TG. Stress-induced activation of Nox contributes to cell survival signalling via production of hydrogen peroxide. J Neurochem 2009; **109:** 1544-54.

84. Ward PS, Thompson CB. Metabolic reprogramming: a cancer hallmark even warburg did not anticipate. Cancer Cell 2012; **21:** 297-308.

85. Collet JF, Stroobant V, Van Schaftingen E. Mechanistic studies of phosphoserine phosphatase, an enzyme related to P-type ATPases. J Biol Chem 1999; **274:** 33985-90.

86. Shelley EJ, Madigan MC, Natoli R, Penfold PL, Provis JM. Cone degeneration in aging and age-related macular degeneration. Arch Ophthalmol 2009; **127:** 483-92.

87. Kern TS, Berkowitz BA. Photoreceptors in diabetic retinopathy. J Diabetes Investig 2015; **6:** 371-80.

88. Hartong DT, Berson EL, Dryja TP. Retinitis pigmentosa. Lancet 2006; **368:** 1795-809.

89. Travis GH. Mechanisms of cell death in the inherited retinal degenerations. Am J Hum Genet 1998; **62:** 503-8.

90. Byrne LC, Dalkara D, Luna G, Fisher SK, Clerin E, Sahel JA *et al.* Viral-mediated RdCVF and RdCVFL expression protects cone and rod photoreceptors in retinal degeneration. J Clin Invest 2015; **125:** 105-16.

91. Fu Y, Yau KW. Phototransduction in mouse rods and cones. Pflugers Arch 2007; **454:** 805-19.

92. Peichl L, Gonzalez-Soriano J. Morphological types of horizontal cell in rodent retinae: a comparison of rat, mouse, gerbil, and guinea pig. Vis Neurosci 1994; **11:** 501-17.

93. Blakeslee B, Jacobs GH, Neitz J. Spectral mechanisms in the tree squirrel retina. J Comp Physiol A 1988; **162:** 773-80.

94. Kryger Z, Galli-Resta L, Jacobs GH, Reese BE. The topography of rod and cone photoreceptors in the retina of the ground squirrel. Vis Neurosci 1998; **15:** 685-91.

95. Long KO, Fisher SK. The distributions of photoreceptors and ganglion cells in the California ground squirrel, Spermophilus beecheyi. J Comp Neurol 1983; **221:** 329-40.

96. Blanks JC, Johnson LV. Specific binding of peanut lectin to a class of retinal photoreceptor cells. A species comparison. Invest Ophthalmol Vis Sci 1984; **25:** 546-57.

97. Hendrickson A, Hicks D. Distribution and density of medium- and short-wavelength selective cones in the domestic pig retina. Exp Eye Res 2002; **74:** 435-44.

98. Nikonov SS, Daniele LL, Zhu X, Craft CM, Swaroop A, Pugh EN, Jr. Photoreceptors of Nrl -/- mice coexpress functional S- and M-cone opsins having distinct inactivation mechanisms. J Gen Physiol 2005; **125:** 287-304.

99. Daniele LL, Lillo C, Lyubarsky AL, Nikonov SS, Philp N, Mears AJ *et al.* Cone-like morphological, molecular, and electrophysiological features of the photoreceptors of the Nrl knockout mouse. Invest Ophthalmol Vis Sci 2005; **46:** 2156-67.

100. Bobu C, Craft CM, Masson-Pevet M, Hicks D. Photoreceptor organization and rhythmic phagocytosis in the nile rat Arvicanthis ansorgei: a novel diurnal rodent model for the study of cone pathophysiology. Invest Ophthalmol Vis Sci 2006; **47:** 3109-18.

101. Gaillard F, Bonfield S, Gilmour GS, Kuny S, Mema SC, Martin BT *et al.* Retinal anatomy and visual performance in a diurnal cone-rich laboratory rodent, the Nile grass rat (Arvicanthis niloticus). J Comp Neurol 2008; **510:** 525-38.

Figures

Fig 1.

Structural differences between rods and cones. Photoreceptor cells are composed of four structural regions: outer segment (OS), inner segment (IS), cell body (CB), and synaptic terminal (Syn). Cone OS are conical shaped and shorter than rod OS. Cone OS are formed by invaginations of the plasma membrane into stacks of membranous discs (dark grey). The cone opsins exist as transmembrane proteins in these membranous discs. Cone OS discs are connected to a ciliary membrane (orange) that extends the length of the OS. A ciliary membrane also extends the length of rod OS; however, rod OS discs are not connected to the ciliary membrane. Cone IS contain twice as many mitochondria (pink) as rod IS. Glut1 receptors and ion pumps (green) are heavily concentrated in the IS of both types of photoreceptors.

Fig 2.

Suggested model of energy consumption in cones. In darkness, energy is consumed mainly by ion pumps the inner segment. In light, the process of photo-transduction consumes energy in the outer segment. ATP from oxidative phosphorylation is produced by mitochondria in the inner segment to support the ion pumps. ATP from glycolysis is produced in the outer segment to support photo-transduction. ATP is also transported from the inner segment to the outer segment via the cilium and also through a phosphocreatine shuttle system to support photo-transduction.