

The history and physiology of human tritan perception

Dr Ross Littlewood, Midland Eye Clinic, Western Australia

reception@midlandeyeclinic.com.au

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This essay is not a scientific treatise. It is a metanarrative that aims to interweave many complex threads of knowledge in order to provide an accessible narrative synthesis for time pressured readers. Curiosity about colour vision has led me down countless arcane academic byways, but the more I explored them the more incomplete my understanding appeared. A scientific strategy for dealing with missing data is interpolation from what is known, but as a philosopher I am aware that the most accessible narratives are the most valuable, and that truth describes an emergent property of discourse, not a preceding condition. This narrative suits my present purposes, but it may not suite those who wish to avoid approximations, speculations, and simplifications for the sake of narrative synthesis. Those enquiring minds are encouraged to read (1) *Swab IR. Evolution's Witness: How Eyes Evolved. Oxford University Press: New York, USA, 2012 (1)*. I happen to believe the world needs more meta-narratives for the paradoxical reason that it is good for science. The present decline in the respect afforded to scientific evidence within social media is partly due to a misperception that 'science' is a special resource for the epistemologically elite or the time privileged, rather than a practical resource for the time pressured public. It needs to take control of its public image, and accessible meta-narratives are a means to that end.

Among the oldest evidence for life on earth are microfossils discovered in 3.5 Ga (billions of years ago) silicates sandwiched between layers of volcanic basalt in the remote Pilbara region of Western Australia. Paleobiologists believe ancient, microscopic, heat loving organisms found in these rocks, are the oldest known evidence of life on earth. Those organisms belong to the kingdom 'Archaea,' and consist of a single cell with no nucleus. The fossilized Archaea found in Pilbarra were capable of photosynthesis. Surviving species of Archaea have been discovered living around undersea hydrothermal vents. They appear to have evolved separately to bacteria and can survive more extreme chemical and thermal stresses. Perhaps life began around 3.75 Ga when the oceans first formed from condensing gas, or perhaps even earlier if photosynthesis evolved using Hydrogen before water

was widely available. There is no settled consensus, let alone evidence, of when life began or what the first life form consisted of, so a 3.7 Ga Archaea is as good a starting point as any.

It is known that there are several forms of chemical photosynthesis, of which the most ancient is 'C3 photosynthesis.' This chemical reaction is inefficient at high temperatures, but the efficiency can be improved if it occurs in combination with a second method of converting sunlight into energy, using Vitamin A derivatives; provided energy can be exchanged between the molecules. 'Vitamin A' denotes the pro-Vitamin A carotenoids found in plants and their fat soluble derivatives. An ancient form was probably first incorporated into cell walls around 3 Ga to provide structural rigidity, because it contains a long chain of double molecular bonds that provided increased resilience. This chemical structure also confers light absorbing properties, producing visible pigmentation. Following its structural integration it was 'co-opted' to provide an increasingly diverse range of biological functions during evolutionary history. Probably the first was to act as a chromophore in single celled organisms. The long chain of double bonds present in the isoprene subunits in all forms of Vitamin A can readily capture solar energy by altering their atomic bonds, and that stored energy can be passed onto other molecules. The peak absorption of Vitamin A is from 500 to 600nm, which happens to be the range over which chlorophyll absorbs very poorly, so the combination of chlorophyll with Vitamin A has become ubiquitous in plants. Variations in the ratio of chlorophyll and Vitamin A explain many of the seasonal variations in plant foliage. This combination also occurs in marine organisms, such as phytoplankton, that form the basis of the marine food web. It is likely that ancient single celled marine organisms exposed to UV light in shallow water first began to produce variants of Vitamin A through oxidative cleavage during the proterozoic era (2,500 - 541 Ma), and retinaldehyde and its oxidative products have since become ubiquitous in the light sensing organs of all animal species.

The terminology of photochemistry is complex, so before proceeding with this narrative it is necessary to digress into the topic of photochemical nomenclature. When 19th century anatomists dissected animal eyes they observed a rose pink colour in the retina that bleached on exposure to light. They named this substance 'rhodopsin' because they were good at ancient Greek, not because they were good at organic chemistry (Rhodon = rose, Opsin = sight). Subsequent chemical analysis showed that what was called rhodopsin was a group of related chemicals that varied between organisms. The chemical responsible for the colour change was always A1, or 11-cis retinal in vertebrate eyes, but in other species it was sometimes a different Vitamin A derivative - A2, A3 or A4. Some species use more than one type of

rhodopsin, for example fish that migrate from a short wavelength lighting environment in open water into a longer wavelength environment in shallow streams can convert A1 into A2 using an enzyme named CYP27C1. This conversion produces a red shift in the spectral sensitivity of all their photoreceptors. Other organisms have also evolved this adaptation to changing light called the 'rhodopsin–porphyropsin' switch (since A2 is a porphyrin). In all cases 'rhodopsin' referred to one or other Vitamin A derived chromophore bound to an opsin protein in an anatomical retina, not to the chromophore alone. Some authors make the mistake of referring to a Vitamin A derived chromophore in solution as rhodopsin, but it is legitimate to refer to rhodopsin as being constituted of two subunits, an opsin apoprotein and the retinal chromophore (2).

The earliest use that single cells made of the light absorbing properties of Vitamin A was to use the structural change in all-trans retinal(dehyde) to open and close a membrane pore, or 'ion channel'. Opening or closing a channel in a membrane allows organisms to accumulate charged particles on one side - called 'pumping protons.' 'Proton pumps' still exists in some modern single celled organisms, including cyanobacteria and a protist named *Euglena Gracilis*. The single celled organism *Chlamydomonas reinhartii* contains a different chromophore to retinal called CRY2 which is also found in the intrinsically photosensitive retinal ganglion cells (ipRGC) of the human retina. It is therefore certain that proton pumps evolved more than once. One common use of the energy was to move a 'flagellum' or "tail" to produce mobility in response to light. It is a short evolutionary step from using light to move a flagellum to using light to control the direction of movement. This was achieved by shielding one side of the receptor with a light blocking pigment (3). Before chromophores were used for vision they became widely distributed as solar powered energy sources.

By the time the first multicellular organisms appear in the fossil record, a little over 600 Ma, all-trans retinal was widely distributed amongst eukaryotic cells (those with a cell nucleus as distinct from 'prokaryotic' cells without a nucleus). As well as 'proton pumps' various specialized membrane structures evolved that were capable of 'switching' between alternate states in response to an external stimulus. Often this process involved binding to a chemical transmitter, or ligand, resulting in electron transfer. These structures are widely distributed in vertebrates and are called 'G protein coupled receptors' (GPCR). They have their own evolutionary history (4). The best known GPCR is rhodopsin, which undergoes a 'switch' from one 3-dimensional shape to another when activated by light. Collections of GPCR's on the surface of an organism are called placodes, and these collections further increased the organism's sensitivity. The next evolutionary step was a

change in the retinal chromophore from 'all trans retinal' found in unicellular organisms and invertebrates, to 11-cis retinal (A1). A significant consequence of this change is that all-trans retinal is 'bistable' but 11-cis retinal is 'monostable.' This means that all-trans retinal can be regenerated by light in situ through photobleaching, but 11-cis retinal must be released from its binding site after conversion to all-trans retinal by the absorption of light, and then undergo an energy dependent enzymatic process to restore the 11-cis version of the molecule. Therefore the necessary enzymes and receptors for re-synthesis also needed to evolve (5). (Zhong M, Kasaguchi R, Kassai M & Sun H. Retina, Retinol, Retinal and the Natural History of Vitamin A as a light sensor. *Nutrients* 2012;4(12):2069-2096).

It is becoming obvious that many of the evolutionary steps towards a modern primate eye will not be anatomically visible in the fossil record, including the evolution of hormones and neural circuits. Other evolutionary stages such as alterations in the shape and location of the primitive eye, the surrounding pigment, and surface covering, have left some traces in the fossil record. The specialised neural tissues of the ancestral eyes and their neural connections both germinated from the same embryonic stem cells called ectodermal cells. The first neural connections were probably a mere 'tangle' of nerve fibres, but eventually organised networks evolved. It is important for this narrative to note that the early development of a specialised control centre for orientating vertebrates towards the light occurred at a time when short wavelength detection was the dominant requirement for survival. The evolutionary drift from ion pumps using all-trans retinal to high concentrations of 11-cis retinal in specialised receptors with attached neurons occurred over a span of hundreds of millions of years.

Vertebrate 11-cis retinal is found in combination with a small protein called an opsin. The earliest known opsin evolved around 700 Ma and is found in marine Placozoans, but they do not use it to absorb light. A variety of opsins have since evolved, not all of which detect light, but the group known as ciliary have evolved to do so. Some of these C-opsins increase the light absorbing efficiency of retinal by up to three times compared to pure retinal in solution. They also narrow the wavelength range over which 11-cis retinal is most responsive - called 'spectral tuning.' Although they are integral to how light energy is converted into a chemical and electrical response in modern eyes their evolutionary story is rather incomplete. There is evidence that the earliest C opsins were tuned to short wavelengths and that rod and long wavelength opsins evolved later. Among the 'non visual opsins' are some located in the retinal pigment epithelium that preferentially bind all-trans retinal after it has been isomerized from 11-cis retinal by photo transduction. Each primate pigment epithelial cell is responsible for "re-

charging” up to 30 overlying photoreceptors with reconstituted 11-cis retinal. The first image forming complex eye in the fossil record (as opposed to the compound eye of insects and crustaceans) belonged to a trilobite, an extinct species of marine arthropod found in fossils dating from 530 Ma discovered in South Australia. The species survived until an extinction event around 250 Ma and it presumably possessed the necessary enzymes to convert all-trans retinal into 11-cis retinal. It is likely that the evolutionary benefit of combining retinal with opsin was realised before the ‘Cambrian explosion’ 540 Ma, which preceded the ‘Vertebrate land invasion’ in the late Devonian period (~440 Ma), since both land and marine animals employ this combination.

The pre-Devonian vertebrates had one opsin for rods and four for cones - two short wavelength sensitive opsins (SWS1 and SWS2) a mid wavelength opsin similar to rod opsin (Rh2), and a long wavelength sensitive opsin (LWS). Each opsin has a different but overlapping spectral response curve and their absorption maxima have changed during evolutionary history. The most adaptive absorption maximum for an aquatic ‘pre Devonian’ vertebrate without an efficient optical system for concentrating light was the 350nm to 550 nm range because shorter wavelengths interact more efficiently with chromophores underwater; navigation in the absence of image formation involved use of direct rather than indirect light sources; and it was critical to avoid intense short wavelength light below 300 nm because it denatures proteins. After complex eyes evolved and light detection became more efficient it was increasingly adaptive to avoid physical hazards, so a co-ordinated response to a change in the lighting became important. The need to co-ordinate reflex behaviour by multicellular organisms produced the selection pressure that enable a neural network called the tectum to evolve. The tectum is still highly conserved in all vertebrates, including primates, and its reflex behaviour is still governed by changes in short wavelength luminance and tritan (blue-yellow) contrast. It controls an organisms explicit response to light including eye movements and pupil reactions. There is a separate but interconnected circuit that controls the circadian rhythm. As the tectum was evolving there were two SWS opsins and the range of detectable light energy extended below 400nm, into the range we now call ‘ultraviolet’ because we can no longer detect it. Although there is a striking homology among the opsins of terrestrial vertebrates there is a butterfly with 16 opsins, a shrimp with 12, and even the tiny Zebra fish has four cone opsins whereas humans have three.

Zoological science was long concerned with discovering the fossilised evidence that would explain the physical evolutionary steps in the development of the eye (as opposed to the process of seeing). That was the part of the evolutionary narrative that engaged Charles Darwin. He found the

micro-evolutionary steps so difficult to elucidate that he delayed the publication of 'The Origin of Species' (1859) for several years while he worked on the puzzle. He wrote "To suppose that the eye, with all its inimitable contrivances.... could have been formed by natural selection, seems, I freely confess, absurd in the highest possible degree," but in 1993 two Swedish zoologists published a theoretical model which requires 1829 micro-evolutionary steps to produce a complex eye from an eye spot. Since the reproductive cycle of progenitor species was predictably short, they concluded that "a few hundred thousand years" is sufficient for evolution of a complex eye (6). Many developmental zoologists and paleobiologists now consider it unproblematic to account for the phenotypic stages required for a complex eye to evolve, despite large gaps in the fossil record.

The Australian zoologist Andrew Parker has proposed an influential 'Light Switch' theory to explain the unprecedented rate of evolutionary development that occurred concurrently with the evolution of complex eyes - a phenomenon called the 'Cambrian explosion' that began 540 Ma. According to this theory competition between species that shared a new found ability to grow image forming eyes rapidly transformed predator species into prey and vice versa, becoming the dominant driver of evolutionary adaptation and selection during that period (7). Evolutionary geneticists have now provided some of the genetic explanations necessary to account for evolution, one of which is the concept of 'master control genes.' They may have developed through fusion of multiple smaller genes, and control a suite of other genes, directing organogenesis. This concept has become important for explaining everything from the axial body plan of complex organisms to the number of wings and legs in a fly. One such 'master control gene' is Pax-6, that controls the development of the complex eye in species from Drosophila flies, fish, and mice to primates and modern humans. It may have evolved from the combination of several precursor genes and is relevant to clinical ophthalmology since a mis-sense mutation in Pax-6 causes aniridia.

As we have seen the vertebrates invaded the land during the late Devonian period (~440 Ma), and at ~ 200 Ma the earliest mammals appear in the fossil record. They were small non-placental creatures, with 11-cis retinal as the chromophore, rod opsin, but only 3 of the 4 cone opsins present in their ancestral vertebrate species. There is some uncertainty about marsupials, but as a general rule they did not retain the Rh2 (mid wavelength sensitive) opsin. The placental mammals (eutherians) first appear in the fossil record ~150 Ma retaining 11-cis retinal (A1) as a chromophore, rod opsin and just 2 of the original 4 vertebrate cone opsins (SWS1 and LWS). Note that the evolutionary trend of reducing opsin variety implies that the prevailing

selection pressures encouraged sensitivity to light energy rather than discrimination between wavelengths. A possible reason for this is that the species that survived were those that could avoid becoming dinosaur lunch by being nocturnal. The terrestrial dinosaurs evolved 240 Ma and the visual acuity of dinosaur species is estimated to have varied from similar to a crocodile, (non stereoscopic and around 6/36) all the way up to similar to a human (stereoscopic vision with at least 6/9 acuity). As the ancestor species of modern primates only survived if they were nocturnal they had rod dominated retinas without a deep foveal pit, and had little use for multiple cone opsins. They had not evolved the retinal signal processing and topographical neural signalling that enables 'seeing' in the sense we understand it now, since they did not have a neocortex. What mattered at this stage of evolution for species survival was the ability to detect changes in the direction or brightness of light with at least equal sensitivity to your predators and other competing species (8).

The next important epoch in this narrative is the Cretaceous-Paleogene (K-T, or K-Pg) mass extinction event ~66 Ma in which a cyclical change in the plane of rotation of the solar system exposed the earth to large number of meteor impacts that reduced atmospheric oxygen and world temperatures and led to a mass extinction that notably involved all non avian dinosaurs. After many climatic fluctuations an even more significant epoch for this narrative occurred, called the Paleocene-Eocene thermal maximum (PETM) ~55 Ma at the start of the Eocene geological epoch. The temperature rose rapidly to 5 to 8 degrees centigrade above modern levels, there was no polar ice, high sea levels, and large amounts of the green house gasses carbon dioxide and methane were in the atmosphere. Throughout the Eocene the oxygen level of the atmosphere increased and the level of greenhouse gasses reduced as a result of plant growth. Despite many species extinctions caused by the rapid and severe climate change during the PETM the fossil record first shows evidence of the precursors of all our modern domestic animals and primates at that time, and the subsequent changes in the climate favoured their survival. This is both significant and paradoxical - given our current climate predicament. An early primate progenitor was Purgatorius, an animal slightly larger than a dormouse, that appeared in the fossil record ~65 Ma. Its skeleton is consistent with an arboreal habit, which may have been a factor in its survival at the time of the K-T mass extinction. By ~50 Ma the oxygen level in the atmosphere had doubled from historical lows of the K-T extinction because of the extent of plant re-growth, but the earth was much warmer than now with sea levels ~80 metres above the current level. Europe and most of Asia were archipelagos. Prosimian (ancestral primate) fossils found in Eurasia from ~50 to ~40 Ma show a progressive increase in the size of the brain and eye and a forward movement of the foramen magnum at the base of the skull, suggesting a

progressively more upright posture and a developing neocortex, consistent with increased reliance on diurnal vision to explore the world and an expanding visual horizon.

This stage in the evolutionary narrative is critical to understanding the way modern primates process spectral data. It marks a time, relatively late in evolutionary history, when long wavelength discrimination became more adaptive than tritan (short wavelength) contrast as a determinant of species survival. The capacity to represent the world through mental processes was not possible until the neocortex evolved, and although the primitive tectal reflexes have continued to function in response to tritan contrast, the world the early primates explored was one where the ability to recognise and grasp objects, and to make distinctions between the colour of leaves at dusk from beneath a tree canopy, were powerful advantages for species survival. The neocortex evolved under these conditions, and the mental representations of the world that came to dominate behaviour were constructed mainly from the receptive fields of long wavelength detecting cones. To know both 'where it is' and 'what it is' requires a higher level of processing than the ability to respond reflexively to changes in luminance, since it requires the construction of an internal representation of the "it" to which those properties inhere. During its evolution the neocortex developed a few connections with the midbrain, such as accommodation, but they involve relatively small numbers of neurons. For the most part the newly developing light processing pathways and 'world modelling' capacities of the neocortex were "bolted on top of" the pre-existing primitive brain circuits.

Prosimians had a more rod dominated retina and less stereoscopic vision than later primate species. The prosimian population became divided ~40 Ma into two strictly separate groups living on each side of the Atlantic Ocean - the 'Old World Primates' (catarrhine lineage) of Africa and Eurasia, and the 'New World Primates' (platyrrhine lineage) of South America. Those continents rifted around 140 Ma but were closer together at 40 Ma than now, and it has been suggested that some ancestral prosimians may have crossed a now eroded land bridge in the Atlantic Ocean, or floated part of the way on a raft of vegetation. Regardless of their means of transport the ancestral Prosimian population divided into two subpopulations that have since remained physically and genetically distinct ~40 Ma. At that point the "wild" LWS gene on the end of the X chromosome of ancestral Prosimians had several variants. These included LWS genes with amino acid substitutions at three or more locations, resulting in a "green" LWS variant with a spectral peak at 530 nm instead of the usual 560nm, a 'yellow' variant with fewer substitutions, and others. Similar variations of the LWS gene are still found in modern Primates and humans. Expression of the LWS

gene is controlled by an adjacent 'Locus control region' (LCR) located 'upstream' (away from the terminal end) on the X chromosome.

After the ancestral Prosimian lineage divided the genetic heterogeneity of LWS persisted in both groups, allowing for a statistically infrequent co-occurrence where a single female who carries two very different variants of the LWS gene in each of her two X chromosomes could express them alternately in different cells across her retina to create a 'mosaic' form of trichromacy (since according to the Lyon hypothesis both sexes produce the same amount of X chromosome products because females, with double the number of X chromosome genes, express only one per cell). This heterogeneity enabled a limited form of trichromacy that remains relevant in the 'New World Primates, but the 'Old World Primate' lineage later acquired a more reliable type of trichromacy of benefit to both sexes that is therefore more adaptive. Amongst the 'Old World Primates' of Asia and Africa the fossil record is most interesting in Egypt, which was forested ~35 Ma. Two separate groups of primate fossils have been recovered from the Fayoum deposit there, including an early progenitor of all 'Old World Primates' (monkeys, apes, and humanoid species). The ancestral 'Old World Primates' encountered a localised version of climate change along a north to south rift through eastern Africa, called the rift valley. This is important because sedimentary accumulation in the valley has preserved the remains of those that moved into that region long ago as fossils, providing much of the information we now have about the early origin of man.

We have arrived at ~35 Ma and the stage is now set for the appearance of full primate trichromacy, but first it is necessary to make another digression. Using genetic engineering missing opsin genes have been inserted into the retina of visually mature dichromatic squirrel monkeys with a subsequent change in their ability to respond to a colour contrast task (9). This altered ability is not the same as 'seeing' an extra colour, or being able to precisely match it, and it is certainly far short of having the ability to match the perception to a socially determined semantic system. Nevertheless it may still be surprising that an adult animal could have any change in colour vision capacity after such an intervention since each retinal cone already had a mature set of connections to specific neurons in the brain. Perhaps this is because 'seeing' for a squirrel monkey does not mean making human-like internal representations of an external reality in a Cartesian sense. 'Seeing' is polysemic, so in some Primates it may simply mean exploring the world using visually specific sensorimotor paradigms in which perceptions are incomplete and subject to varying perspectives. Nevertheless there is abundant evidence that the primate brain has enough 'neuroplasticity' to utilise new data streams whenever the march of evolution offers those

affordances, and we need to make that assumption for the next change in Primate colour detection to produce a meaningful change in perception (10).

Between 35 Ma and 25 Ma 'Old World Primate' species evolved into larger and more diurnal species. As they did so they increasingly utilised long wavelength light for navigation since longer wavelengths produce a sharper retinal focus in long eyes. It is an optical fact that scattering and dispersion occur far more among short wavelengths than long ones along any extended light path, in the absence of a vacuum. The opening up of savannah grasslands around the rift valley also favoured the evolution of a deeper foveal pit, since it magnifies the central 1 degree of visual field. Both changes emphasised the evolving advantage of long wavelength detection compared to short wavelength detection at the foveal centre. Given these conditions a further enhancement of long wavelength discrimination was likely to be detectable. It is in this context that a fateful genetic 'accident' occurred during the early stages of meiosis in a species that was ancestral to later Old World Primates. Meiosis is the process through which genetic mixing through sexual reproduction is possible. In each future sexual partner the genetic content of one 'diploid' cell is reduced by half to produce two haploid cells (eggs in ovary or sperm in testes). The blending of this material and reconstitution of one diploid cell from the mixture of genetically different haploid cells is the basis of sexual reproduction. The early stage of meiosis requires the paired strands of each chromosome separate, and then to line up and exchange genetic material. Sometimes incomplete separation and unequal division of genetic material occurs, and in the X chromosome this occurs most commonly at the terminal end. When the exchange results in a large but damaged copy of the LWS gene from one strand becoming attached to the terminal end of the normal LWS gene a 'tandem array' of two LWS variants has been produced. This is the 'genetic accident' that occurred at some time before 25 Ma and became established as a stable variant by that time. Such events are not rare, and in fact there are human X chromosomes with 4 LWS gene variants at their terminal end. A newly minted MWS gene was not the only necessary change. Some alteration in the locus control region was also necessary so that in each cone cell it would be possible that one or the other gene would become active. Once this occurred full primate trichromacy was born ~25 Ma. This arrangement has endured, but as the MWS gene now occupies the terminal location it is more vulnerable than the LWS gene to further 'genetic accidents.' This explains the relatively high frequency of anomalous MWS genes in humans (~6%).

Receptor trichromacy (SWS, MWS, and LWS opsins) is a necessary but not a sufficient condition for altered colour perception. Full primate trichromacy

also requires retinal receptive fields that use opponent processing to compare the LWS cone signal with the MWS cone signal, as well as other receptive fields that compare the SWS cone signal with the average of LWS and MWS ('yellow') signals. Beyond that it also requires sufficient 'neuroplasticity' to create a novel perception from the novel stimulus information. The remarkable neuroplasticity of the primate neocortex is frequently overlooked when considering our evolutionary story. This genetic story has been elegantly told by Nathans in many publications including (11) *Jacobs GH & Nathans J. Color Vision: How Our Eyes Reflect Primate Evolution. Scientific American April 2009*, and is more complicated than what I have presented here. Full trichromacy evolved separately in an ancestor of one species of New World Primate, the South American howler monkey, around 15 Ma (12). This shows that convergent evolution is more common than we might suppose.

Fossils from East Africa dated > 11 Ma were the progenitors of the Great apes, and our earliest hominid ancestors left evidence in the fossil record dating from > 7 Ma. During the Pleistocene epoch, from 2.5 Ma to 11,700 years ago, the earth's climate cycled from glacial to interglacial, and the East African environment changed to produce even more open savannah grasslands. This favoured larger primates with longer legs and better long distance visual acuity. It also encouraged socialisation which provided survival advantages for those groups who were most co-operative. The increasing axial length produced retinas with fewer and fewer blue cones in the central fovea, and in modern humans the central foveola is entirely blue cone free (13). The absence of blue (SWS) cones in the central fovea can only be demonstrated under experimental conditions, using small field monochromatic lights or band pass filters. This is because what we perceive as 'visual consciousness' is an abstraction from physical reality, not a direct translation of it (14).

The scattering of blue light onto the MWS and LWS cones can activate them, due to the principle of invariance (any receptor will respond to any wavelength if it is sufficiently strong in precisely the same way as it would to the wavelength to which it is targeted). When scattered blue light 'fogs' the output of the more spatially informative long wavelength cones in the central fovea it interferes with their contrast detection. This is the reason rifle sights often have yellow (blue blocking) filters. The image magnification created by the foveal pit only benefits resolution between wavelengths that have not been scattered over many receptors. It also improves vernier acuity (15). The high information density in the optical and long wavelength receptor signals within the foveola is maintained at the neural level by midget ganglion cells with very small receptive fields that transmit the retinal signal without loss of

spatial resolution. Their axons comprise the parvocellular system that dominates our visual consciousness, conveying both the luminance contrast necessary to form a mental representation of the world, and also the red/green contrast most useful for attributing secondary properties to objects. The parasol ganglion cells have larger receptive fields with input from rods and both MWS and LWS cones. They respond to movement or changes in luminance and can redirect the attention of the parvocellular system. Their large axons comprise the magnocellular ganglion system. Abnormalities in the magnocellular system are associated with dyslexia and schizophrenia. Both the parvocellular and magnocellular fibres project to the lateral geniculate nucleus where they remain distinct as their signals are modulated by descending cortical fibres. A further cell body projects the modulate signal directly to the primary visual cortex (area V1).

Groups of blue cones form the central receptive field of bistratified ganglion cells that convey tritan (blue/yellow) contrast. The yellow surround of the receptive field is formed from the addition of MWS and LWS cones and rods. There is some controversy about whether or not the centre is always 'blue on' or whether there are bistratified ganglion cells in primates that genuinely have a 'blue off' central field, as opposed to merely a 'yellow on' surround. The bistratified ganglion cells comprise the koniocellular system (16). They have large receptive fields but small axons that are histochemically distinct from the parvocellular and magnocellular fibres (17) They are also more vulnerable to damage in a range of disease than the parvocellular and magnocellular fibres, and unlike the parvocellular and magnocellular systems the koniocellular system exerts no direct control over mental processes. The relative sub-populations of ganglion cells among the 1.7 million optic nerve fibres is 80% parvocellular, and 8% each of magnocellular and koniocellular. Of the remaining 4% the most interesting are the intrinsically photosensitive retinal ganglion cells (ipRGC's) that contain the chromophore melanopsin and respond directly to light with a spectral maximum of 480 nm (18). This group of fibres are arranged in two layers in the inner retina, and they also receive some input from the SWS cones. They project to the hypothalamus where they entrain the circadian rhythm and stimulate release of melanopsin. They influence pupil size and the rate of firing of cells in the LGN in mice, but their role in human vision is less well understood (19). There is no evidence that they contribute directly to our experience of colour vision or luminance. A further 13 types of retinal ganglion cells have been described in primates (20).

The koniocellular system is actually a set of subsystems due to its long and complicated evolutionary history. Like the parvocellular and magnocellular systems it also projects to discrete layers in the lateral geniculate nucleus,

but unlike them it also projects to many other brain areas. At the lateral geniculate nucleus (LGN) the 'bottom up' koniocellular signal is spared the modulation that occurs in the parvocellular pathway. This pathway specificity is important to understanding amblyopia, since where tritan contrast is not affected. The three major pathways remain anatomically and functionally discrete all the way to V1 where the "tritan" koniocellular sub-system terminates among cell columns at a different level to the parvocellular fibres. Another koniocellular sub-system projects directly to visual association areas, bypassing the primary visual cortex and so not contributing to tritan contrast perception. Most of this projection terminates in area V5 (17). This pathway is thought to be the basis of 'blind sight' (18,19). The middle temporal area (V5) is concerned with tracking movement and responds mostly to luminance contrast rather than chromatic contrast (20). The other important koniocellular sub-system is the highly conserved tectal pathway.

There is no unity of perception in V1 - it is a 'parallel processor.' Electrodes placed in V1 in primates do not respond discretely to any of the four unique hues, whereas electrodes placed in the posterior temporal cortex do. It is presumably only the 'tritan' koniocellular sub-system, projecting to V1, that contributes to conscious tritan perception, so a test for 'tritan discrimination' is not a test of the 'koniocellular system' as such. We have an anthropomorphic tendency to theorise backwards from our position of unified perception and assume that 'seeing' always entails the unified experience that we are privileged to have by virtue of a highly evolved neocortex. 'Seeing' in the sense we experience it is a parvocellular experience, albeit directed by the magnocellular system and refined just prior consciousness by the addition of socially normative colour categories - which may be the stage of colour processing where the koniocellular and parvocellular pathways are finally unified.

The perception of colour is an emergent psychological process out of physiologically constrained red/green and blue/yellow opponent processes, as well as black/white opponency. The primary colours of red, green, blue and yellow are unique hues in that unlike every other hue they can exist perceptually without any sense of admixture. There is one unique green that appears to contain no blue or yellow. The unique hues plus black/white contrast can be mixed to produce all the secondary colours. A modern scientific approach to measuring colour using these physiological principles is the Swedish 'Natural Colour System' (NCS), but virtually all previous systems were non physiological. This includes Newton's attempt to force a system of 7 divisions onto our colour perceptions so as to ensure it corresponded with the 7 divisions in the musical scale. Other widely used colour ordering systems such as the Munsell system from America and

various industrial standards, such as the Pantone system, have specific uses. What is remarkable is how predictably humans convert the linear variation of wavelength into a colour opponent perception. The predictability of this process has allowed the International Commission on Illumination (CIE) to define various 'standard observer' models that reliably predict the perception that will result from mixing component hues. The results vary with pupil size since the receptor array is non uniform in the ratio of cone types and ganglion cell receptive fields.

As language developed over the last 150,000 years or more each culture developed a similar system for categorising and naming colours (24). However languages only provide limited conceptual and linguistic categories for users to deploy when naming their colour perceptions, and this leads to the problem of linguistic relativism. English provides 11 categories, Russian 12, but Wobe only 3. Languages with a reduced colour vocabulary tend to evolve colour names in a non random sequence, beginning in all cultures with red and in almost all followed by green and yellow. This should not be surprising given how our parvocellular system dominates consciousness, and it is also not surprising that the category most frequently missing a colour name is blue. Those languages that offer an extra colour name not available in English usually refer to a subdivision of the english 'blue.' I should not ignore this opportunity to point out how powerful the paradigm of colour vision is at 'illuminating' the ancient philosophical debate about perception and essence. The world of 'physical colour stimulus' is undeniably linear, but our world of 'conscious colour perception' is undeniably circular - as illustrated by a colour wheel in which green and red are opposite and red blends into purple the blue.

Tritan contrast in clinical practice.

Clinicians have rarely measure tritan contrast in the past for a number of reasons, including the fact that it was usually difficult to find a reliable test that was time and cost efficient. There is also an age related reduction in tritan contrast due to the selective absorption of short wavelengths by the ageing lens nucleus, and most accessible inexpensive 'colour tests' were those designed to detect whether the red/green contrast was normal or abnormal. In some cultures it matters quite a lot whether a young male refers to their environment using the standards of colour reference that are normative. Inherited anomalies of the MWS or LWS cone are most often expressed in males as they have only one chance of carrying a normal gene because they have only one X chromosome. Needing to know this was once

important in military service, and has been considered important for occupational reasons, but it is not informative about the health of a person's visual system. Acquired disease of the retina and optic nerve usually impact tritan contrast more than red/green contrast and asymmetrical loss of tritan contrast is not only a more reliable disease indicator but less likely to recover than acquired loss of red/green contrast. The C test for tritan contrast is a 10 step tritan contrast test that requires less time to administer than an Ishihara test, and has been validated under as providing a reliable ordinal score from 0 to 10 under sufficient lighting. It remains useful even in older adults with significant cataract (25). This is useful in several clinical situations, including:

- (a) *Amblyopia* - selective suppression of the parvocellular signal at the LGN means the C test is normal. This can be clinically useful (26).
- (b) *Retinal vascular diseases* in which both retinal oedema and ischemia occur effect the parvocellular and koniocellular systems differently. Oedema has relatively less impact on tritan contrast but ischemia has relatively more. Since the prognosis for recovery is better following oedema than ischemia a low C test score is prognostically useful. Even high altitudes cause reduced blue cone ERG amplitude (27), and the fragile koniocellular fibres are also more vulnerable to disease than the thicker and histochemically distinct parvocellular fibres.
- (c) *Optic neuropathies* of all types tend to affect tritan contrast early and it is less likely to recover than acquired red/green defects. The widely used Ishihara plates differ in difficulty more through non-colorimetric than colorimetric differences (number size, differences in crowding, and differences in plate design) and have a colorimetric dynamic range less than a third of the C test plates. Tritan contrast measured by the C test is an exclusively colorimetric measure whereas the particular 'parvocellular puzzle' presented by the Ishihara plates is not purely colorimetric. The colorimetric dynamic range within the C test is more than 3 times that of the Ishihara plates, and the test is quicker to administer. An important caveat it that the C test is more demanding than the Ishihara with respect to adequate lighting and correct test procedure, but when those conditions are met it produces reliable and repeatable results.

The C test for tritan discrimination is designed for clinicians. It is available on line at ctest.com.au, but there are many more sophisticated tests of koniocellular function that are better suited for colour vision research.

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