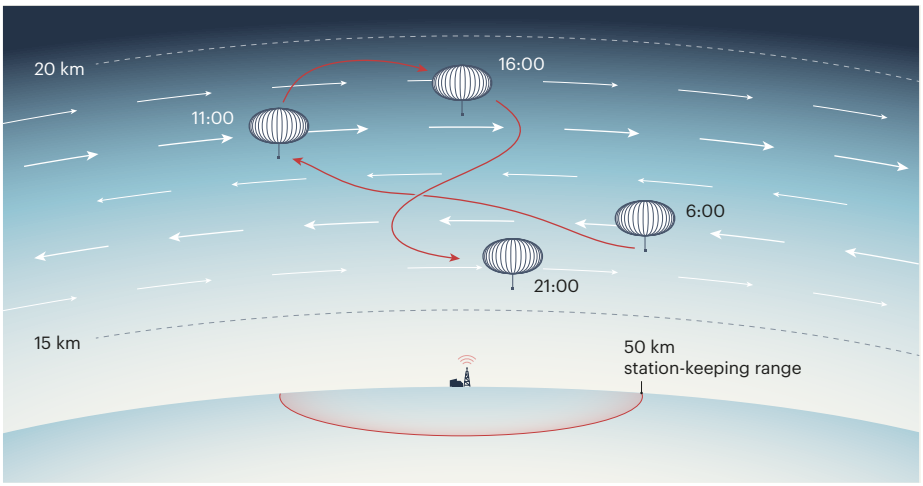

Figure 2 | Station-keeping for stratospheric balloons. Unmanned balloons, known as super-pressure balloons, are used to carry out experiments in the upper atmosphere. Station-keeping is the act of maintaining the position of a balloon within a certain range of a specific position on the ground. The balloon's height is altered during the day to move it between altitudes at which winds blow in different directions – when the balloon is driven away from its station by winds at one height, it moves to a different height at which the winds can blow it back again. Bellemare *et al.*[2] report that a type of machine learning, known as reinforcement learning, can be used to train an autonomous control system for station-keeping that outperforms previously used control systems.

and battery health. Bellemare and co-workers' success therefore represents a big advance in the use of reinforcement learning for real-world applications.

Station-keeping performance is ultimately limited by the range of wind speeds and directions in the region surrounding the balloons (at heights of 15–20 kilometres, for the current study). The winds must also switch direction so that balloons can adjust their trajectory to stay within range of the station. These special conditions only persist for months at a time within the Equatorial stratosphere, where Bellemare and colleagues' study was carried out – and where a slow procession of opposing winds peak in strength near 30 km, before descending and dissipating near 15 km, switching direction every 14 months or so[5].

Such wind diversity also occurs elsewhere, but is less reliable and generally occurs beyond the range of heights at which a single super-pressure balloon can operate. During the flight campaign described in the current study, larger wind disturbances originating from high latitudes occurred in the tropical stratosphere, and probably assisted station-keeping. Bellemare and colleagues' system might therefore struggle to achieve the same success at other locations. However, smaller, more rapid wind variations can also occur, including atmospheric waves of various types[6], which a skilful controller could navigate to its advantage.

The advent of effective autonomous super-pressure balloons would open up a range of commercial and scientific applications for probing Earth's atmosphere and that of other planets. Such balloons are already used to study small and large-scale waves in the tropical stratosphere[7], and to detect low-frequency sounds produced by the ocean[8], lightning[9] and earthquakes[10]. They have also been proposed for use in future explorations of Venus's atmosphere[11], to search for signs of active volcanism and chemical signatures of life[12]. Moreover, the ability to fix a balloon's geographical position is crucial if balloons are to be used to build an aerial wireless network for telecommunications – an early objective of Project Loon, the owners of the balloons used in Bellemare and colleagues' study.

Station-keeping a balloon for months at a time would allow long-term environmental monitoring, for example, of air quality over cities, of carbon fluxes from heat-stressed forests and of regions of thawing permafrost. Other applications include monitoring animal-migration routes and illicit trafficking of goods and people across borders. These applications will become increasingly relevant as the effects of climate change become more pronounced, as restrictions on movement are imposed by global events such as COVID-19, and as long-term climate-change mitigation involving aviation prompts the search for alternative platforms for making aerial observations.

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Ageing

Sight restored by turning back the epigenetic clock

Andrew D. Huberman

Neurons progressively deteriorate with age and lose resilience to injury. It emerges that treatment with three transcription factors can re-endow neurons in the mature eye with youthful characteristics and the capacity to regenerate. **See p.124**

Ageing has negative consequences for all the cells and organs in our bodies. Our brains are no exception. Neurons in the developing brain form circuits that can adapt to change and regenerate in response to injury. These capacities have long been known[1] to diminish over time, but the molecular shifts that underlie this deterioration have remained mysterious. Lu *et al.*[2] show on page 124 that neurons of the eye can be programmed to revert to a youthful state in which they reacquire their ability to resist injury and to regenerate. The authors' findings shed light on mechanisms of ageing and point to a potent therapeutic target for age-related neuronal diseases.

Retinal ganglion cells (RGCs) reside in the eyes and thus outside the skull, but they are bona fide brain neurons. They initially develop

as part of the forebrain. Subsequently, RGCs extend projections called axons out of the eye to make connections with neurons in the brain itself. These axons — which join together to form the optic nerve — survive and regenerate if they are damaged early in development, but not after they reach maturity[3,4]. Evidence indicates[3,5] that this shift is intrinsic to RGCs, rather than reflecting changes in the surrounding cells.

Myriad studies have searched for factors that can prevent or promote RGC survival and regeneration. A handful of such factors have been identified that can endow mature RGCs with some degree of survival and regenerative capacity — but not enough to fully maintain or restore vision after damage to the optic nerve[4].

Lu *et al.* asked whether it is possible to revert RGCs to a younger 'age', and whether doing so would allow the cells to regenerate. They infected RGCs in mice with adeno-associated viruses. These harmless viruses had been genetically engineered to induce expression of three of the 'Yamanaka factors' — a group of four transcription factors (Oct4, Sox2, Klf4 and c-Myc) that can trigger mature cell types to adopt an immature state[6]. Such an approach normally comes with hazards *in vivo*: Yamanaka factors can cause cells to adopt unwanted new identities and characteristics, leading to tumours or death[7]. Fortunately, Lu and co-workers found that they could circumvent these hazards by expressing just Oct4, Sox2 and Klf4 (together called OSK).

The authors tested the infected RGCs' ability to regenerate if the cells' axons were crushed. They found that the OSK-expressing viruses triggered RGC regeneration and long-distance axon extension following damage to the optic nerve (Fig. 1), with no apparent alterations to RGC identity, formation of retinal tumours or any other ill effects.

OSK expression had beneficial effects on RGC axon regeneration in both young and aged mice. In some cases, the regenerated axons extended all the way from the eye to the optic chiasm (the location at the base of the brain at which the optic nerves from each eye cross to the opposite brain hemisphere). It is notable that the effects of OSK are seen in older animals, because studies of RGC regeneration are often conducted in relatively young animals, which have a residual natural regenerative ability. Thus, the evidence suggests that Lu and colleagues' approach can fully restore long-distance regenerative capacity in mature RGCs — a milestone for the field.

Almost all techniques previously used to enhance RGC survival and axon regrowth had to be performed before optic-nerve damage[4] — a restriction incompatible with using a technique therapeutically. Excitingly, Lu and colleagues showed that they could induce OSK expression at different time points — even after axon injury — and still improve RGC survival and regeneration. These effects were not limited to optic-nerve injury; OSK expression also effectively reversed RGC and vision loss in a mouse model of glaucoma (the most common cause of human blindness). Expression of OSK in RGCs after axon and vision loss (but before the RGCs died) fully restored vision in these animals. The same was true for wild-type old mice: OSK allowed old mice to regain youthful eyesight.

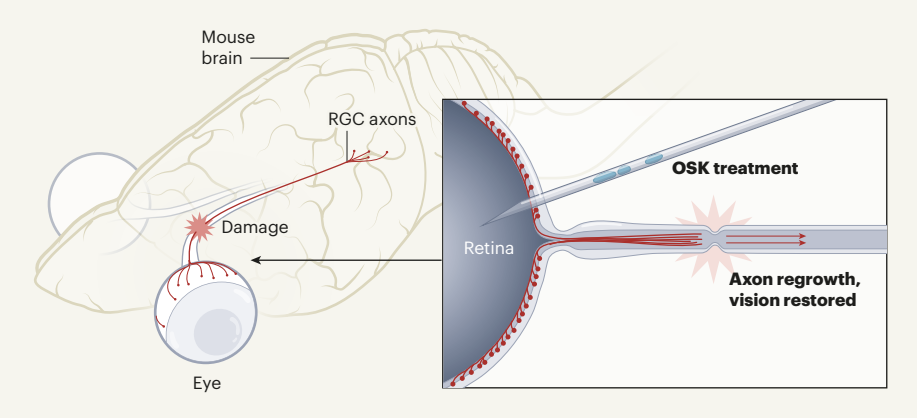

Figure 1 | Restoring vision in mice. Retinal ganglion cells (RGCs) transmit visual information from the eye to the brain along projections called axons. Damage to the RGC axons prevents transmission of this information, leading to sight loss. Lu *et al.*[2] report that treatment of damaged RGCs with a transcription-factor cocktail called OSK restores the cells to a youthful state, leading to axon regeneration and restoration of sight in mice.

Why might reprogramming old RGCs to a younger state promote regeneration and restore vision? An emerging model in the field of ageing is that, over time, cells accumulate epigenetic noise — molecular changes that alter patterns of gene expression[8], including transcriptional changes and shifts in the patterns of methyl groups on DNA. Collectively, these changes cause cells to lose their identity and so to lose the DNA-, RNA- and protein-expression patterns that once promoted their youthful resilience[9,10]. Given the growing excitement about DNA methylation as a marker of cell age, the authors asked whether OSK expression somehow counteracts the negative effects of ageing or axon injury on DNA methylation.

> **"Expression of the OSK transcription factors allowed old mice to regain youthful eyesight."**

The RNA components of a cell's protein-synthesizing machine, called the ribosome, are encoded by ribosomal DNA genes that steadily accrue methyl marks with age. The ribosomal 'DNA methylation clock' is therefore considered to be a reliable estimate of cell age[11]. Lu *et al.* found that damaging the axons of RGCs accelerated ribosomal DNA methylation in a way that mimicked accelerated cellular ageing, whereas OSK expression counteracted that acceleration, indicating that tissue injury in general might be a form of accelerated ageing.

The group also tested whether the removal of DNA methylation is required for OSK to regenerate axons or restore vision in old mice. The TET enzymes (TET1, TET2 and TET3) catalyse the removal of DNA methylation[12]. The authors showed that OSK induced expression of *TET1* and *TET2* genes, and that reducing TET1 and TET2 production blocked the effects of OSK on RGC regeneration and vision restoration in old mice. Thus, changes in DNA methylation seem essential for the effects of OSK. Indeed, Lu *et al.* found that OSK restored youthful DNA-methylation patterns across a broad set of genes involved in neuron survival, outgrowth and connectivity. These patterns occur at chromosomal regions that have high levels of PRC2 — a protein complex that alters methylation during development and ageing[13]. Going forward, it will be important to determine the exact extent to which the positive effects of OSK are mediated by resetting DNA-methylation patterns, and the downstream mechanisms that guide the cellular reset.

Are Lu and colleagues' findings likely to be relevant to humans? The authors found that OSK expression enhanced axon regrowth and cell survival in human neurons *in vitro*. The effects of OSK in people remain to be tested, but the existing results suggest that OSK is likely to reprogram brain neurons across species.

Future research should also address whether OSK expression can have the same remarkable effects on neurons elsewhere in the brain and spinal cord. Given that RGCs are bona fide brain neurons, there is good reason to think they will. As such, the current findings are bound to ignite great excitement,

not only in the field of vision restoration but also in those looking to understand epigenetic reprogramming of neurons and other cell types generally. For decades, it was argued that understanding normal neural developmental processes would one day lead to the tools to repair the aged or damaged brain. Lu and colleagues' work makes it clear: that era has now arrived.

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Clinical research

Building a chemical blueprint for human blood

Patrick H. Bradley & Katherine S. Pollard

What determines the chemical make-up of human blood? Measurement of the contributions of factors ranging from genetics to lifestyle has now identified diet and gut microbes as key predictors of blood's molecular composition. **See p.135**

Our blood transports many chemicals besides oxygen and carbon dioxide. Some of these molecules provide useful indicators of the state of our health. Indeed, measuring such biomarkers is a common feature of clinical blood tests. Other molecules present, such as hormones and drugs, directly affect health by modulating processes such as metabolism and immune responses. On page 135, Bar *et al.*[1] shed light on the factors that affect the recipe for human blood's chemical brew.

The origin of most blood-borne molecules, and why they vary in concentration between individuals, is unknown. The list of possible regulators is long: for any given molecule, diet, drugs, medical conditions and history, genetic variants and gut microorganisms might all have a role. Furthermore, these factors can interact, as is the case for trimethylamine oxide. This molecule, which promotes the artery-narrowing disease atherosclerosis, is generated as a result of the metabolism, by both microbes and their host, of certain dietary compounds that are abundant in red meat[2]. For molecules such as this, which directly affect health, understanding their metabolic regulation might help to yield new clinical treatments.

Bar *et al.* describe their efforts to tackle the question of what factors govern the molecules present in blood. This work requires not only measurement of the many variables potentially involved, but also the use of analytical methods that can capture complexity — such as the interactions between variables — while still ensuring that valid predictions can be made for individuals outside the study population.

The authors began by characterizing blood samples from a group of 491 healthy individuals in great detail. They quantified the molecules in serum — the liquid component of blood that remains after the proteins needed for clotting have been removed. The study participants provided detailed health information, and answered questionnaires about diet and lifestyle. They also gave stool samples, which were used for DNA sequencing, to determine the genetic signatures of the gut microbes present (also known as the microbiome).

As the authors acknowledge, this is a small study group by the standards of genome-wide association studies, which seek to find connections between genes and disease. Bar *et al.* are also not the first to link serum molecules to genetic variation or the microbiome[3,4]. However, the authors' analysis of this group of individuals is unique in the number of data types that were systematically collected to investigate serum composition.

Next, Bar *et al.* used a machine-learning approach to link factors such as human genetics and microbiome information to the molecules in the blood. By carrying out many analyses omitting different data subsets, the authors found that diet, the microbiome and clinical variables such as prescription-drug use and blood pressure had the most associations with serum molecules. Although the authors found some genetic associations, confirming 46 previously reported gene–metabolite links, they concluded that the association effects for genetic factors were smaller than were those for diet, clinical variables and the microbiome. These various data types are not exactly comparable, but the authors' estimates of the genetic effects are in line with results from

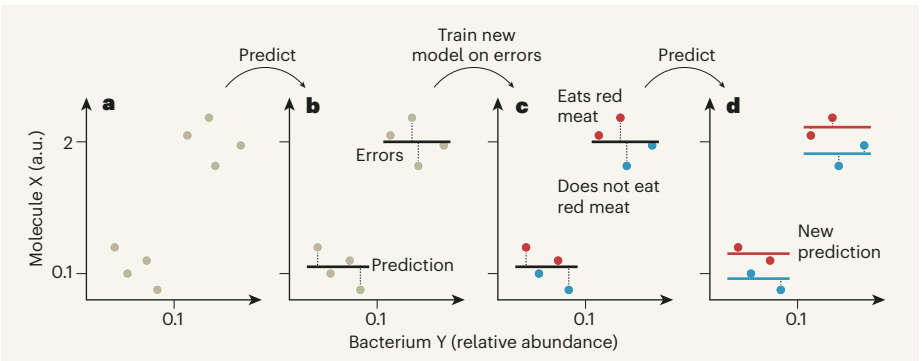

Figure 1 | A way to predict blood's molecular composition. Bar *et al.*[1] obtained human blood samples and identified many of the molecules present. The authors also gathered information about a range of factors, such as diet and gut microbes, that might have affected the molecules found. Using a computational method called gradient-boosted decision trees, Bar and colleagues predicted the molecular composition of an individual's blood. **a**, In this hypothetical example, data points show an individual's concentration of molecule X in arbitrary units (a.u.) and the relative abundance of a type of gut bacterium Y. **b**, The model uses an 'if–then' classification to predict (black horizontal lines) the relationship between bacterial abundance and the concentration of X. The prediction in this case is that if the bacterial abundance is more than 0.1, the concentration of X is 2, and if this abundance is less than 0.1, the concentration of X is 0.1. Dotted lines show the prediction errors. **c**, The model is then refined by taking into account another factor, such as whether the person eats red meat (red) or not (blue). **d**, After another 'if–then' classification that includes this dietary factor, the model generates refined predictions (red and blue horizontal lines) with lower errors that link the predicted concentration of X to dietary and bacterial factors.