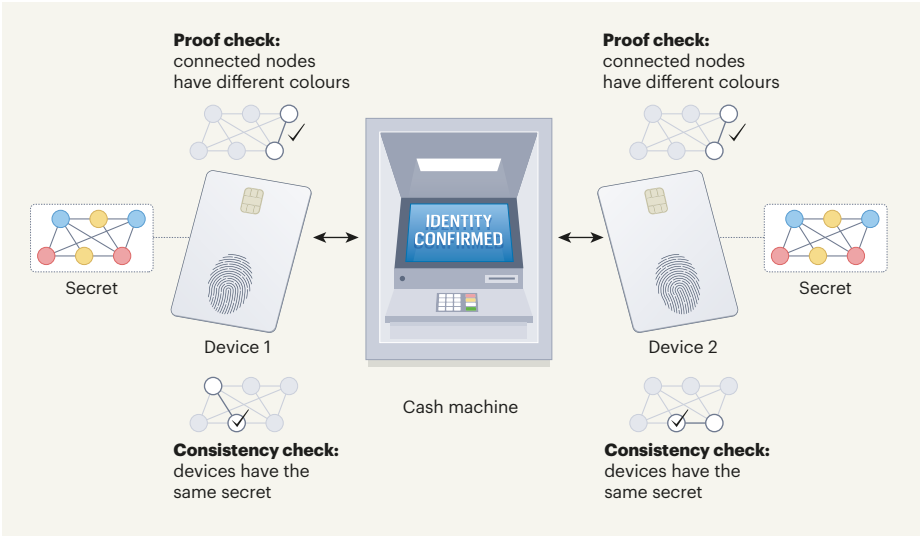

Figure 1 | An identification scheme using special relativity. Cash machines could perform identity checks that are based on the solution to a puzzle, such as the network used by Alikhani and co-workers[1]. Two devices share secret information that tells them how to colour a network's nodes so that no two connecting nodes have the same colour. The user activates the devices with a thumbprint, and the cash machine chooses two connected nodes and asks the devices to show that they have different colours. Answers are encoded so that the actual colours are revealed only when both devices are asked about the same node, and the colours are changed around to generate different solutions of the network puzzle between questions. These measures prevent fake machines from learning information to impersonate the user. Random 'trap' questions verify that the two devices' secret solutions are consistent. Repeating this process many times will expose fake devices, which would either assign the same colour to connected nodes or fail the consistency test. Limiting the time allowed for answers prevents the devices from helping each other to cheat, a consequence of Einstein's special theory of relativity.

more economical scheme[7] was proposed in 2020, which brings us to the work featured in Alikhani and co-workers' study.

The authors describe two implementations of a simplified version of the economical scheme proposed last year. In one experiment, the devices were placed at either end of a building at the University of Geneva in Switzerland and were separated by 60 metres — a distance that light takes 200 nanoseconds to travel. This is clearly an unreasonable distance to use in the cash-machine scenario, because it would require Alice's devices to be 60 metres apart. However, the authors argue that improvements within the reach of current technology would allow them to reduce this distance to only one metre. It is therefore possible to imagine Alice sliding her biometrically activated smart cards into two slots of the cash machine. It still might not be the most practical solution, but it is on the right track.

In addition to offering a promising approach to the conundrum of a secure cash machine, the work by Alikhani and colleagues succeeds in demonstrating a feasible relativistic zero-knowledge proof of a non-trivial mathematical statement. However, there are two theoretical caveats. First, the puzzle that Alice would be using to prove her identity could be solved by a malicious party that has sufficient computing power. And second, it might become possible for fraudsters to defeat the identification scheme by harnessing quantum entanglement[8], the mysterious property of quantum states that Einstein referred to as "spooky action at a distance". Although special relativity prevents the two devices from communicating quickly enough to fool a cash machine into mistaking them for Alice's devices, entanglement might allow the right correlations to appear instantaneously between the cheating devices with no need to know the puzzle's solution[9].

It remains to be seen whether this could compromise the identification process[10]. More-complicated schemes could defeat all possible quantum attacks[7,11], but they are currently beyond the reach of practical implementation. Further work is needed — both theoretical and technological — before these ideas can find their way to your local bank.

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Metabolism

A hormonal two-step to drive physical activity

Stephanie L. Padilla

In mice, the ovarian hormone oestradiol sensitizes neurons in a brain region called the hypothalamus to a melanocortin hormone that signals an energy surplus. Their dual activation increases physical activity. **See p.131**

Mammals become less physically active with ageing, and, in females, this decline in activity is tied to reproductive ageing. After menopause, women tend to be less active and to develop increased total fat mass and altered fat distribution[1]. In rodents, surgical removal of the ovaries (ovariectomy) reduces the levels of ovarian hormones such as oestradiol in a similar way to the effects of menopause, and results in reduced physical activity and corresponding increases in weight. On page 131, Krause *et al.*[2] identify a population of neurons in the ventrolateral subdivision of the brain's ventromedial hypothalamus (VMH) that are sensitive to oestradiol. These neurons regulate the balance between sedentary behaviour and physical activity on the basis of perceived energy state, with menopause and the associated reduction in oestradiol causing a shift towards sedentary behaviour.

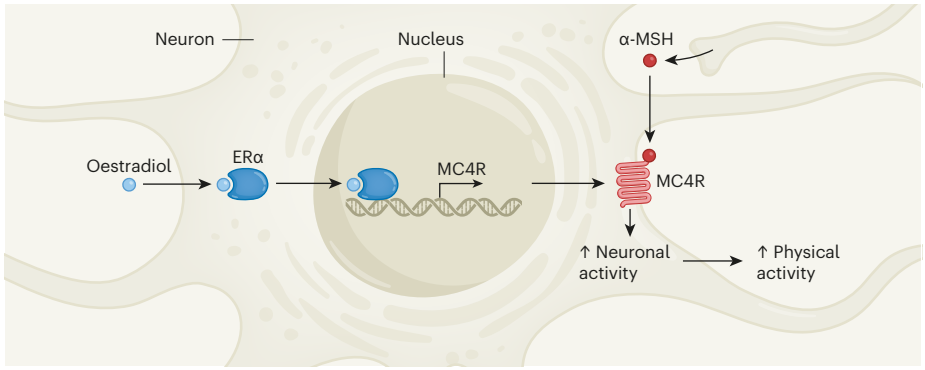

Figure 1 | A two-part signalling mechanism to promote physical activity. The ovarian hormone oestradiol promotes physical activity, and menopause and surgical removal of the ovaries are associated with an increase in sedentary behaviour and weight gain. Krause *et al.*[2] investigated the mechanism by which oestradiol drives physical activity in female mice. By signalling through the oestrogen receptor-α (ERα), oestradiol increases expression of the gene that encodes the protein melanocortin 4 receptor (MC4R) in neuronal cells of a part of the brain called the ventrolateral subdivision of the ventromedial hypothalamus. The melanocortin hormone α-melanocyte stimulating hormone (α-MSH), which is produced when there is a surplus of energy in the body (when energy supplies or stores are in excess of the body's energy demands), signals through the MC4R to increase the activity of these neurons, driving an increase in physical activity and thus energy expenditure.

Oestradiol has emerged as the ovarian hormone that has the greatest effect on energy expenditure in females. Its levels in the body fluctuate over the course of the oestrous cycle (the recurring pattern of changes in ovarian activity), with low amounts circulating in the blood during what is known as the oestrus phase of the cycle and high amounts during the proestrus phase. Oestradiol binds to, and thus signals through, the oestrogen receptor-α (ERα) protein to promote energy expenditure through physical activity and the generation of heat (thermogenesis)[3]. The authors suggest that, with the decline in oestradiol levels after menopause, neurons in the ventrolateral VMH become less responsive to melanocortin hormone signalling that serves as a metabolic indicator of energy surplus (when energy supplies or stores are in excess of the body's energy demands), and are consequently tuned to drive sedentary behaviour.

Krause *et al.* profiled the molecular dynamics of oestradiol signalling in the ventrolateral VMH of female mice of reproductive age across the animals' oestrous cycle. They found that levels of oestrogens circulating in the blood correlated with gene expression in the ventrolateral VMH. Treating ovariectomized mice with oestradiol increased the expression of many genes involved in metabolism in these neurons, including the *Mc4r* gene, which encodes the melanocortin 4 receptor protein. It is known that the melanocortin hormone α-melanocyte stimulating hormone (α-MSH) binds to and signals through MC4R to promote satiety and energy expenditure in response to an energy surplus[4]. In other words, melanocortin signalling promotes energy use when nutrients or energy stores are abundant.

The increase in gene expression in ventrolateral VMH neurons that is associated with increased oestradiol was not accompanied by a rise in the activity of these neurons. However, treatment of mice in the oestrus phase with oestradiol, together with a drug that activates MC4R, markedly increased the activity of these neurons. This intriguing finding hints at a stepwise activation cascade, whereby oestradiol and melanocortin signalling are needed in succession to activate ventrolateral VMH neurons (Fig. 1). By driving *Mc4r* expression, oestradiol might tune these neurons to become more sensitive to input signals that promote energy use.

Krause *et al.* found that nearly half of ERα-expressing neurons in the ventrolateral VMH also express MC4R, and thus could show this stepwise response (hereafter dubbed 'stepwise' neurons). Beyond its role in metabolism, the ventrolateral VMH is also a node in a network of brain regions that regulate social behaviour. Specifically, ERα-expressing neurons in the ventrolateral VMH control aggression and mating behaviours[5]. The authors' finding of a molecularly distinct subset of ERα-expressing neurons that also express MC4R raises the possibility that certain metabolic and social outcomes rely on subpopulations of ERα-expressing neurons in the ventrolateral VMH.

The authors next investigated the role of these stepwise neurons in metabolism. The VMH and, in particular, ERα signalling in this brain region are implicated in the control of energy expenditure[6]. For example, previous work showed that blocking ERα expression, and thus artificially reducing oestradiol signalling in the ventrolateral VMH, in female rodents resulted in overt body-weight gain and sedentary behaviour[7]. Because MC4R-expressing neurons are restricted mostly to the ventrolateral subdivision of the VMH, and form a subset of the ERα population, Krause *et al.* used genetic tools targeting MC4R-expressing cells to manipulate the stepwise neurons.

The authors genetically engineered mice such that the animals' stepwise neurons could be activated by the injection of a small molecule called CNO. One such injection rapidly induced an episode of extremely high physical activity, with CNO-treated mice travelling 700 times farther in a 5-hour period than control mice did! Furthermore, mice that consumed CNO in their drinking water over eight days showed considerable weight loss. The CNO treatment did not affect thermogenesis by brown adipose (fat) tissue or the animals' ability to handle increases in blood glucose (their glucose tolerance), suggesting that the weight loss caused by CNO treatment was due to increased physical activity.

Notably, the physical activity of mice that consumed CNO over an extended period was not restricted to the night time (when mice, as a nocturnal species, are usually most active). Instead, these mice seemed hyper-aroused, engaging in physical activity in the day time, when mice usually rest. Consistent with this, the authors found that the stepwise neurons send projections to regions of the brain that regulate escape behaviours and arousal.

Artificially inhibiting the stepwise neurons in non-ovariectomized mice reduced the animals' physical activity. Because sedentary behaviour is associated with the loss of ovarian hormones, including oestradiol, the authors tested whether enhancing the activity of stepwise neurons could protect against the increase in sedentary behaviour caused by ovariectomy. Indeed, in ovariectomized mice, artificial activation of the neurons using CNO increased the animals' activity levels and induced weight loss within a mere 24 hours of treatment.

In a particularly intriguing experiment, the authors used a gene-editing technique called CRISPRa to overexpress *Mc4r* in the ventrolateral VMH of ovariectomized mice (as has been done in mice lacking one of the two copies of the *Mc4r* gene, to curb obesity[8]). If reductions in *Mc4r* expression in stepwise neurons is responsible for the sedentary behaviour after ovariectomy or menopause, then restoring *Mc4r* expression should promote physical activity and drive weight loss.

The authors found that CRISPRa-mediated *Mc4r* overexpression was sufficient to promote physical activity in non-ovariectomized mice. And, unlike in the CNO experiments, the physical activity induced by *Mc4r* overexpression was restricted to the normal active phase at night. In this experiment, the authors short-circuit the stepwise neurons to restore sensitivity to melanocortin signalling. Because α-MSH production is biased to the active phase, it is not surprising that increases in physical activity were also restricted to the

appropriate active phase. Unfortunately, however, overexpressing *Mc4r* in the ventrolateral VMH was not sufficient to restore the physical activity or body weight of ovariectomized mice to pre-ovariectomy levels. This perhaps hints at changed production of α-MSH in the absence of ovarian hormones.

The symptoms of menopause can be highly disruptive and can persist indefinitely. Reduced physical activity and changes in body composition coincident with menopause are also sometimes associated with social stigma. In this work, Krause and team elucidate an oestrogen-dependent neural mechanism that might well impose more-sedentary behaviour. Understanding the downstream brain regions and signalling molecules of the stepwise neurons in the ventrolateral VMH could provide insight into potential pharmacological targets to treat symptoms of menopause.

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Imaging

A view to a cell

Jason R. Swedlow & Lucy Collinson

Efforts to generate nanoscale-resolution images of cell interiors have gained ground through the development and refinement of a microscopy method. The data sets are publicly available as resources for further discoveries. **See p.141 & p.147**

One long-sought goal of cell biology is the full and complete determination of the structure and composition of a single cell. If we could name every molecule that makes up a cell, know each molecule's location, map how the molecules interact, and understand how they come together to construct organelles and determine organelle function, we would have the building blocks needed to understand cellular physiology. Xu *et al.*[1] (page 147) and Heinrich *et al.*[2] (page 141) present combined advances in data acquisition and machine-learning (ML)-based analysis in microscopy that move us closer to achieving this objective.

The development of new technologies or the adaptation of existing tools from other domains have often driven crucial progress towards determining cellular architecture and composition. For example, an imaging technique called focused ion beam-scanning electron microscopy (FIB-SEM) was used for decades in materials science before it was repurposed for the biosciences[3].

In this approach, a series of consecutive images are acquired from biological samples embedded in resin. The method entails cycles of, first, imaging the sample's surface using an electron beam, and then milling away a thin layer of the sample to reveal a new surface for imaging. The resulting data reveal exquisite architectural detail of cells and tissues with a 3D resolution better than 10 nanometres. This is sufficient to clearly distinguish most of the organelles in cells from humans and other species. As a result, FIB-SEM (used alongside other techniques for volume electron microscopy such as serial block-face SEM and array tomography) has opened a window on the inner workings of organs and organelles alike, driving a new field called connectomics (the study of the connections between brain cells to build wiring diagrams of the brain)[4–7], and a surge in the number of studies reporting high-resolution 3D reconstructions of subcellular structures and organelles[8].

The two new papers present major advances in FIB-SEM image acquisition and data analysis that are tailored to the demands of biological research. Xu and colleagues report on the culmination of a multi-year effort to improve the spatial resolution and reliability of FIB-SEM systems, which they achieved by redesigning microscope hardware and monitoring systems to deliver fast, stable, high-resolution imaging over periods of months or years. The team further increased the possible speed of data collection by using a bank of microscopes running in parallel, echoing the use of banks of FIB-SEM systems for device analysis and characterization in the semiconductor industry. The result is spectacular imaging data that reveal the complex 3D constitution of whole cells, with individual organelles clearly visible (Fig. 1).

Xu *et al.* present data for different types of entire cell, including HeLa cells (a line of human cervical cancer cells), T cells of the immune system, and pancreatic β-cells (which secrete the hormone insulin needed to control blood glucose levels). These various cell types were imaged at a resolution of 4 nm in each dimension, meaning that the data can be 'resliced' and viewed from any angle, enabling the viewer to follow cellular membranes across a distance of many micrometres of cell space. Although other imaging methods can achieve higher resolution over smaller volumes, these 3D whole-cell nanoscale atlases will undoubtedly provide a treasure trove of buried information that should offer a deeper understanding of the fundamental processes of life.

With these data in hand, Heinrich and colleagues report the application of artificial intelligence, in the form of advanced ML-based tools, to automatically identify the subcellular organelles that these images reveal. But this is more than just another AI-driven application. The density of data in these new FIB-SEM reconstructions of cells is so great that it exceeds the capacity of manual annotation as a way to define the identity and boundaries of all the cellular constituents — a technique called segmentation. Heinrich *et al.* extended approaches used in several deep-learning-based organelle segmentation tools[9–12] to automatically identify the boundaries of some of the organelles contained in these huge data sets. Their methods are based on the detailed manual annotation of organelle boundaries by human observers in small subsections of the data. These human-annotated data are used as training sets for the ML-derived networks that are then applied to the whole-cell data. This analysis has produced some of the most-detailed models of the architecture of the interior of single cells ever generated.

Their data enabled the authors to measure the volume of individual organelles and the fraction of the total cell volume they inhabit, as well as to identify interesting organelle–organelle interactions; for example, the number of different organelles that make contact with a single microtubule and the various interactions between the endoplasmic reticulum and the Golgi complex. By training their models on annotated data sets from several types of cell, the authors show that generalizable models of cell organelles can be used for organelle segmentation in different cell types. We don't know exactly what features the trained models recognize, but their ability to recognize such features