and each technique requires an energy source to sustain the motion. Furthermore, all the techniques can potentially damage the materials, which reduces the repeatability of each process. This is a problem, because viable nanotechnology must be reliable, reusable and cost-effective to run.

To find a method that fulfils these requirements and that has no need for external intervention, Chang *et al*. set up a series of virtual experiments using computer simulations. This approach offers some advantages over real-world experiments: one can be sure that the materials are free of defects and impurities, and that they are electrically, thermally and chemically isolated. Such isolation is particularly important when seeking intrinsic phenomena.

In their simulations, the authors first laid a nano-flake of graphene on top of a continuous graphene substrate, taking care to ensure that the lattices were not aligned. Graphene is a hexagonal lattice of carbon atoms, only one atomic layer thick, but its structure at the highly reactive edges varies depending on how the edges have been cut[6]. In the simulations, the flake was rotated by 30° with respect to the substrate, so that its edges did not line up with the lattice beneath. This reduced the risk of the flake becoming permanently attached to the substrate as a result of strong covalent bonding between the edges of the flake and the edges of the substrate.

Once it was in position, the authors made no further changes to the flake, but applied a stiffness gradient to the substrate, ranging from 0.801 to 4.005 newtons per metre along one direction. When the molecular-dynamics simulation was engaged, the nano-flake moved spontaneously — from a standing start — from the soft side to the hard side of the substrate (Fig. 1). Then, when it reached the end of the substrate, it rebounded because of a retraction force that pulled it back. Similar forces have been observed to pull extruded cores of multi-walled nanotubes back into the nanotubes[7].

Another advantage of computer simulations is that it allows animations of modelled processes to be made. In the present case, the overall motion is dramatically displayed in an animation provided with the paper's supplementary material. One can see that the graphene flake accelerates as it approaches the hard side of the substrate, and decelerates as it rebounds towards the soft side. This is clear evidence that the stiff side is energetically preferred.

Such stiffness-guided directional motion (termed durotaxis) was first observed in living cells, which also prefer rigidity[8]. Although the biological mechanism for durotaxis in cells remains a mystery, it has another similarity to the nanodurotaxis observed by Chang and co-workers in their simulated system: in both cases, weak van der Waals interactions are present, and in the latter case they were found to be crucial.

To prove this, the authors systematically repeated their virtual experiments under different simulation conditions, varying temperatures, stiffness gradients and stiffness configurations. The results unambiguously showed that the strength of the effective van der Waals potential — the interaction between the flake and the substrate — was inversely proportional to the stiffness. Lower potential energies are always more stable than higher ones, so this explains why the flake moves towards a rigid spot on the substrate: by doing so, it adopts a thermodynamically preferred state. At this stage, it is unclear whether perturbations to the system could be devised to reverse the motion, driving the flake back to soft regions.

Chang and colleagues' findings could have great potential in nanodevices, in part because the observed motion is conveniently unidirectional, but also because the underlying forces fall within a useful and technologically accessible range. The driving force for the nanoscale locomotion is about 320 kilopascals per square nanometre for a 6-nm-wide nanoflake on a stiffness gradient of 0.801–2.403 N m^{-1}. This is not too dissimilar from the forces in biological systems, such as the traction force per unit area exerted by a living cell on a substrate[8,9] and the driving force generated in a protein biomotor[10].

The challenge now is to fabricate graphene substrates that contain deliberate patterns of soft and hard regions, so that the experiments can be recreated in the real world. This will undoubtedly be difficult. It might also be possible to do this for other nanomaterials, but whether the simulated mechanism of nanodurotaxis will work for materials other than graphene is unknown. Nevertheless, the effort is certainly warranted, because strategic patterning of substrates might enable more-complicated trajectories to be realized, opening up new opportunities in nanoscale science and technology. ■

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NEUROSCIENCE

A cellular basis for the munchies

How does marijuana cause the irresistible hunger pangs known as the munchies? Paradoxically, the answer seems to involve an unusual mode of activation of a brain circuit best known for suppressing appetite. SEE ARTICLE P.45

SACHIN PATEL & ROGER D. CONE

According to data gathered by the United Nations[1], 177 million people around the globe use marijuana. So some of you might be familiar with the munchies — that inexplicable drive to eat, stimulated by the active ingredients of marijuana, the cannabinoids. This connection has already led to the development of dronabinol, a synthetic version of the natural cannabinoid Δ-9-tetrahydrocannabinol, as a treatment for the metabolic disorder cachexia anorexia syndrome. But how, and where in the brain, do cannabinoids work to stimulate food intake? In this issue, Koch *et al.*[2] (page 45) report that, when given in doses meant to simulate the effects of marijuana, cannabinoids surprisingly activate a subset of pro-opiomelanocortin (POMC) neurons, a cell group in the brain's hypothalamus that has a central role in inhibiting hunger.

Previous work[3] has shown that ablation of POMC neurons, mutations in the gene encoding the POMC protein, and mutations in the melanocortin 4 receptor (MC4R) in downstream cellular targets of POMC neurons, all cause severe overeating (hyperphagia) and obesity. Conversely, experimental stimulation of the POMC cell group produces a slow-onset inhibition of food intake[4,5]. Cannabinoids bind to receptors dubbed CB_1Rs (for cannabinoid

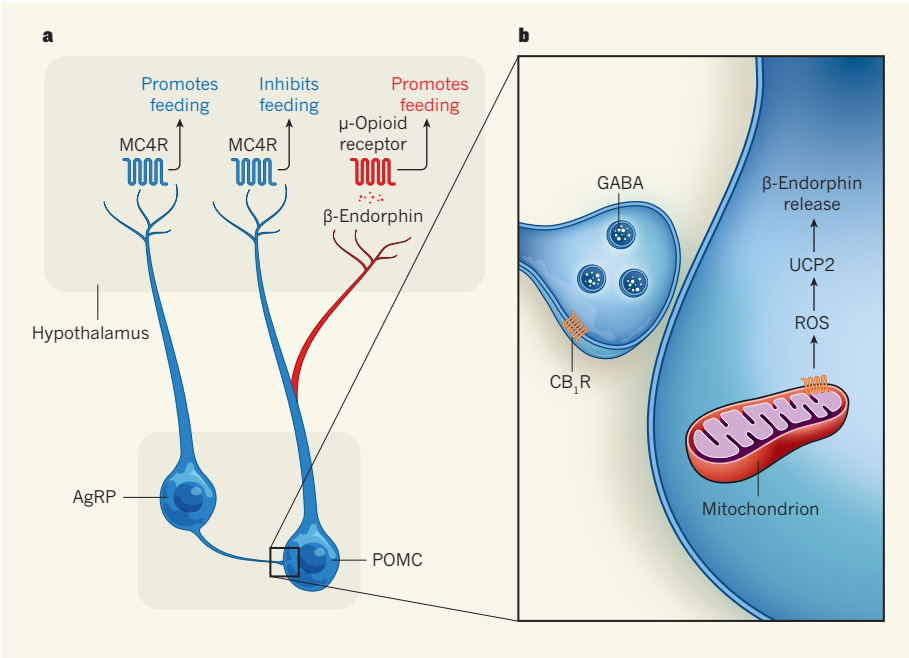

Figure 1 | Cannabinoid regulation of feeding circuits. **a**, The conventional view of appetite circuits in the brain's hypothalamus involves Agouti-related protein (AgRP) neurons, which stimulate feeding, and pro-opiomelanocortin (POMC) neurons, which inhibit it. Both groups affect downstream neurons expressing melanocortin 4 receptors (MC4Rs). Koch *et al.*[2] propose an alternative mechanism, in which cannabinoids stimulate food intake by causing a subset of POMC neurons to specifically activate β-endorphin-releasing boutons (release sites, not shown), which target downstream neurons expressing μ-opioid receptors. **b**, The authors suggest that, by activating cannabinoid receptor 1 (CB_1R) at two sites, cannabinoids increase feeding behaviour. At one site, they could inhibit release of the neurotransmitter GABA from AgRP neurons onto POMC neurons, thereby enhancing the latter neurons' excitability. At the other site, CB_1R activation in mitochondria could increase respiration, production of reactive oxygen species (ROS) and expression of mitochondrial uncoupling protein 2 (UCP2), which then acts as a switch to cause β-endorphin release selectively onto downstream neurons.

receptor 1), and Koch and colleagues observe that, in mice, these receptors are found on nerve terminals that make synaptic connections not only to POMC neurons, but also on organelles called mitochondria, in POMC neurons themselves. This binding stimulates the specific release of an orexigenic (appetite-stimulating) neuropeptide called β-endorphin from the neurons, while somehow avoiding release of α-melanocyte-stimulating hormone (α-MSH), an appetite-suppressing peptide found in these same neurons (Fig. 1a).

That cannabinoids can act at several brain regions to stimulate food intake is well established[6–8]. So the striking lesson here is not so much the orexigenic effect of cannabinoids through yet another of the many brain circuits involved in feeding behaviour. It is rather that cannabinoids can subvert an appetite-inhibitory (anorexic) circuit to become orexigenic, which indicates that the POMC circuit may be even more complex than previously thought. Another challenging concept arising from the present work is that, during the acute orexigenic response, cannabinoids stimulate these neurons partially through intracellular CB_1Rs, rather than through the more usually observed actions of cannabinoids at nerve terminals to regulate the release of substances such as the inhibitory neurotransmitter GABA.

It is noteworthy that, at a neuroanatomical level, the POMC circuit is quite complex, with POMC neurons belonging to the arcuate cluster of cells sending axonal projections to more than 100 brain regions. To add further complexity, a subset of these neurons responds to the hormone insulin; a different subset is affected by the hormone leptin[9], and roughly half of the cells may undergo inhibitory autoregulation by expressing the receptor MC3R (ref. 10). Moreover, almost all POMC neurons produce not only β-endorphin but also α-MSH.

These neurons may therefore be differentially secreting neuropeptides and neurotransmitters to either suppress or stimulate appetite. Parenthetically, administration of analogues of γ-MSH, another peptide released by POMC neurons, also has orexigenic effects[11], suggesting that β-endorphin may not be the only product of POMC neurons that can stimulate food intake. Thus, the emerging picture of the arcuate POMC system is that of a circuit that can sense a wide array of signals and can then produce highly discriminatory responses through a differentiated set of circuits and molecular signalling mechanisms.

What is remarkable about Koch and colleagues' findings is that cannabinoids seem to stimulate β-endorphin release selectively from POMC neurons. Consistent with this, the authors demonstrate that some 34% of the synaptic boutons, or release sites, on POMC neurons selectively express either β-endorphin or α-MSH.

There is, however, a nagging question about the proposed role of β-endorphin as the main mediator of the orexigenic actions of administered cannabinoids in POMC neurons. A previous study[12] found that deletion of the portion of the POMC gene encoding β-endorphin produces hyperphagia and obesity rather than leanness, as might be expected if the primary role of the natural peptide is orexigenic. This issue can be readily addressed by testing whether there is a decrease in the orexigenic response to cannabinoid administration in mice carrying mutations in β-endorphin, or perhaps in mice lacking the μ-opioid receptor, the target of β-endorphin in downstream neurons.

The present data certainly make a clear case for the striking ability of cannabinoids to stimulate a small subset of arcuate POMC neurons and subsequently to increase food intake. Nonetheless, the precise mechanisms of CB_1R-induced activation of POMC neurons and selective β-endorphin release remain to be fully elucidated.

The authors propose two potential routes by which CB_1R activation could boost POMC-neuron activity to increase feeding behaviour (Fig. 1b). First, low doses of CB_1R stimulators could increase firing in a subset of POMC neurons, most likely by reducing the release of incoming GABA signals that would otherwise dampen the neurons' activity[13]. Thus, these stimulators could modify the balance of excitation and inhibition in this neuronal subset. However, a previous study demonstrated[14] that CB_1R expression on neurons secreting the excitatory signal glutamate, rather than GABA, is required for the hyperphagic response to cannabinoids. Furthermore, increases in the firing rate of POMC neurons as a class reduces food intake[4], and thus cannot explain the hyperphagic effects of cannabinoids. So, the possibility of an alternative mechanism to cannabinoid-induced synaptic activation of a specific subset of POMC neurons requires further investigation.

Koch *et al.* suggest that activation of mitochondrial CB_1R represents just such an alternative. CB_1R activation has been shown to block respiration in mitochondria by inhibiting the cAMP–PKA signalling pathway[15]. The authors now extend these findings to show that low levels of CB_1R activation in fact increase mitochondrial respiration in POMC neurons as well as in neurons of the brain's hippocampus. They propose a signalling pathway involving CB_1R-induced increases in mitochondrial respiration, contact between mitochondria and another cellular organelle called the endoplasmic reticulum, generation of reactive oxygen

species and subsequent increased expression of mitochondrial uncoupling protein 2 (UCP2) — a regulator of both mitochondrial respiration in the hypothalamus and feeding (Fig. 1b). They show that UCP2 is essential for cannabinoid effects on mitochondrial respiration, β-endorphin release in the hypothalamus and feeding responses.

More definitive support for this provocative proposed mechanism could be provided by demonstrating that cell-impermeable inhibitors of CB_1R do not block feeding induced by CB_1R activators. Still unknown are the relative dominance of various CB_1R sites in the central nervous system in the orexigenic action of administered cannabinoids, and the relative importance of cell-surface and mitochondrial CB_1Rs. Regardless of this, Koch *et al.* provide another striking example of the complexity of the POMC circuits, and a new cellular mechanism by which cannabinoids stimulate feeding behaviour. ■

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MOLECULAR BIOLOGY

Signals across domains of life

Signal sequences on messenger RNA that initiate protein synthesis are not thought to be interchangeable between life's domains. The finding that a signal from an arthropod virus can function in bacteria questions this idea. **SEE LETTER P.110**

ERIC JAN

All domains of life, from prokaryotes (archaea and bacteria) to eukaryotes (organisms that include plants, animals and fungi) use the ribosome apparatus to synthesize proteins by translating genetic code carried by messenger RNAs. Although the general steps of protein synthesis are evolutionarily conserved, the way in which ribosomes are recruited to an mRNA molecule differs depending on the specific phylogenetic domain[1]. In this issue, Colussi *et al.*[2] (page 110) reveal the surprising finding that a eukaryotic ribosome-recruiting signal is functional in prokaryotic bacteria, thereby challenging the prevailing dogma that prokaryotic and eukaryotic ribosome recruitment are mutually exclusive.

In prokaryotes, ribosomes are generally recruited by a specific signal on the mRNA called the ribosome-binding site (RBS, also called the Shine–Dalgarno sequence). The RBS positions the ribosome over the AUG start codon, a sequence that initiates protein synthesis[1]. By contrast, eukaryotic mRNAs do not contain an RBS, but instead contain covalent modifications — the 5′ cap at the 5′ end, and a poly(A) tail at the 3′ end — that promote the recruitment of ribosomes and protect the mRNA from degradation. Eukaryotes also use at least 12 protein initiation factors (compared to three in prokaryotes) that help to recruit the small subunit of the ribosome to the 5′ cap and facilitate scanning of the mRNA by the ribosome to find the AUG codon.

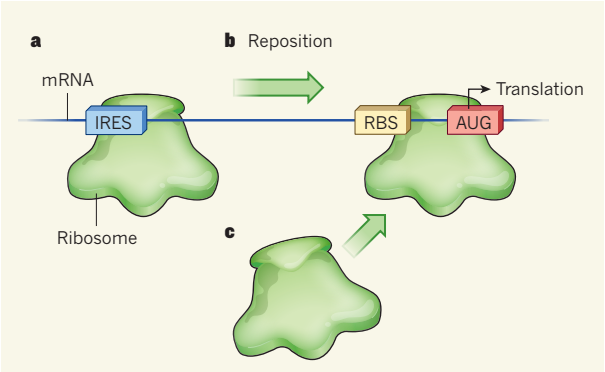

Figure 1 | Internal ribosome entry sites trigger protein synthesis in bacteria. Structures called internal ribosome entry sites (IRESs) in RNA viruses such as the dicistrovirus promote protein synthesis in eukaryotes (organisms including plants, animals and fungi). Colussi *et al.*[2] report the surprising finding that the dicistrovirus IRES can initiate protein synthesis in the bacterium *Escherichia coli* (a prokaryote). **a**, The authors constructed a messenger RNA that incorporates the IRES and introduced this to *E. coli* cells, where the IRES recruits the bacterial ribosome (the protein-synthesizing apparatus) to the mRNA. **b**, They propose that the ribosome then repositions itself to a ribosome-binding site (RBS) and an AUG start codon (an RNA sequence that initiates translation). **c**, Another hypothesis is that, after recruitment of the first ribosome, a second ribosome binds to the RBS and AUG codon.

Some eukaryotic viral RNAs contain alternative signals called internal ribosome entry sites[3] (IRESs) to recruit the ribosome. When host-protein synthesis is inactivated during virus infection, an IRES can bypass normal signals, such as the 5′ cap and some initiation factors, to enlist the ribosome for viral protein synthesis. The simplest known IRES lies within regions found between the genes of dicistroviruses — a family of RNA viruses that infect arthropods[4].

Dicistrovirus IRESs are about 200 nucleotides long and fold into a unique RNA structure. What makes them so remarkable is their ability to bind directly to the ribosome without the need for any initiation factors and to initiate protein synthesis at a non-standard start codon[5] (not AUG). Structural[6] and biochemical studies[7] have shown that part of the IRES structure binds to the conserved core of the ribosome by mimicking a transfer RNA, which normally delivers an amino acid to the growing protein chain. The dicistrovirus IRES has therefore evolved to mimic a component of the normal translational machinery, permitting the viral RNA to hijack the ribosome.

Colussi and colleagues tested whether the dicistrovirus IRES can function in bacteria. To do this, they constructed mRNA that encodes reporter proteins — in this case, luminescent proteins. They found that inclusion of the IRES into the mRNA promotes expression of the reporter proteins in the bacterium *Escherichia coli*. Impressively, the researchers generated a comprehensive set of mutations within the IRES to work out how it functions in bacteria. They observed that some mutations that disrupt the IRES RNA structure