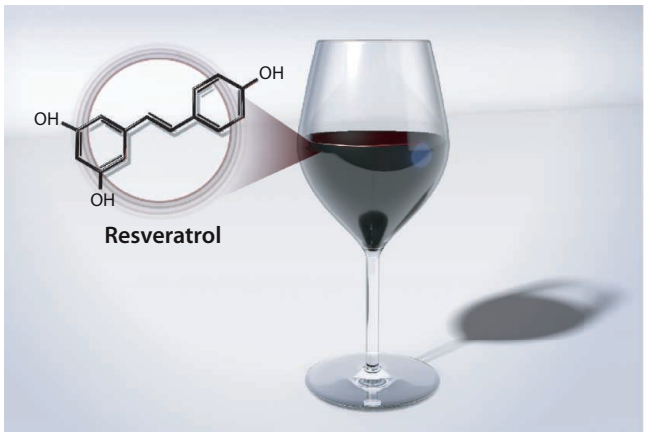

Cheers. Resveratrol, the natural compound in red wine, and other small molecules are allosteric activators of SIRT1, an enzyme with roles in many biological processes (including DNA repair, metabolism, programmed cell death, and inflammation) that affect human life span.

lated on lysine at position 290 (FOXO3a-K290)], as well as other peptides that conformed to this substrate signature, were selectively activated by several STACs. Kinetic analysis of SIRT1 activation by STACs in the presence of these peptide substrates revealed that rate enhancement was mediated primarily through an improvement in peptide binding (lowering of peptide K_M), consistent with an allosteric mechanism of activation. When Hubbard *et al*. screened for SIRT1 mutants resistant to activation by STACs, they identified a single-residue mutation ($Glu^{230} \rightarrow$ Lys; E230K) just to the amino-terminal side of the conserved sirtuin catalytic core. Biophysical studies indicated that in addition to the conserved catalytic core domain and a carboxyl-terminal segment, a small amino-terminal region encompassing Glu^{230} also played a structural role for SIRT1 function. This is consistent with previous studies demonstrating a role for this region in catalysis by SIRT1 (*14*). Moreover, cultured cells lacking endogenous SIRT1 but expressing the mutant enzyme (mouse SIRT1-E222K, the murine equivalent of human SIRT1-E230K) did not respond to several STACs, in contrast to cells expressing wild-type SIRT1. Together, the findings of Hubbard *et al*. demonstrate that STACs can increase the catalytic activity of SIRT1 toward certain substrates through an allosteric mechanism involving an amino-terminal region near the catalytic core, and through direct binding to SIRT1 or a SIRT1-protein complex both in vitro and in cells.

The finding of Hubbard *et al*. has important implications for the further development of SIRT1 modulators. Allosteric activation of SIRT1 through a nonconserved amino-terminal region suggests that SIRT1-selective activators can be developed. Although the current STACs only work against a subset of SIRT1 substrates that contain hydrophobic amino acids at position +1 to the acetylated lysine, this is likely due to the bias of the initial screen that contained a hydrophobic residue mimic (the fluorophore tag) at this position in the substrate peptide. Another screen that is not biased in this way may identify STACs for SIRT1 substrates that contain other sequence signatures. Moreover, if allosteric activators can be developed, then appropriate modifications of these molecules could lead to the development of allosteric SIRT1 inhibitors with comparable protein selectivity. Structural details of the SIRT1 catalytic domain and amino-terminal segment bound to these STACs would facilitate a rational approach to the development of such molecules. That some STACs, like resveratrol, modulate the activity of other molecules (*9*, *15*) is further argument for such structural insights.

There are also implications for understanding some of the seemingly contradictory findings on SIRT1 biology. For example, SIRT1 has been reported to have dual roles—in cell survival with properties of an oncoprotein, and in cell death with properties of a tumor suppressor (*16*). It may be that particular cellular effectors modulate SIRT1 to deacetylate certain protein targets, just as STACs can increase the ability of SIRT1 to deacetylate proteins that contain hydrophobic residues at certain amino acid positions. One could imagine that other stimuli might promote SIRT1 deacetylation of other substrates. Indeed, the amino-terminal segment of SIRT1—the region critical to activation by STACs—might be an important regulatory switch for SIRT1 function, consistent with the ability of the amino-terminal segment of SIRT1 to potentiate the enzyme's deacetylase activity (*14*). Along the same lines, a carboxyl-terminal segment of SIRT1 has also been shown to be important for optimal SIRT1 activity and may have an important regulatory function (*14*, *17*). One thing is now clear—SIRT1 activation by STACs has made a comeback, a renewed reason to toast.

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NEUROSCIENCE

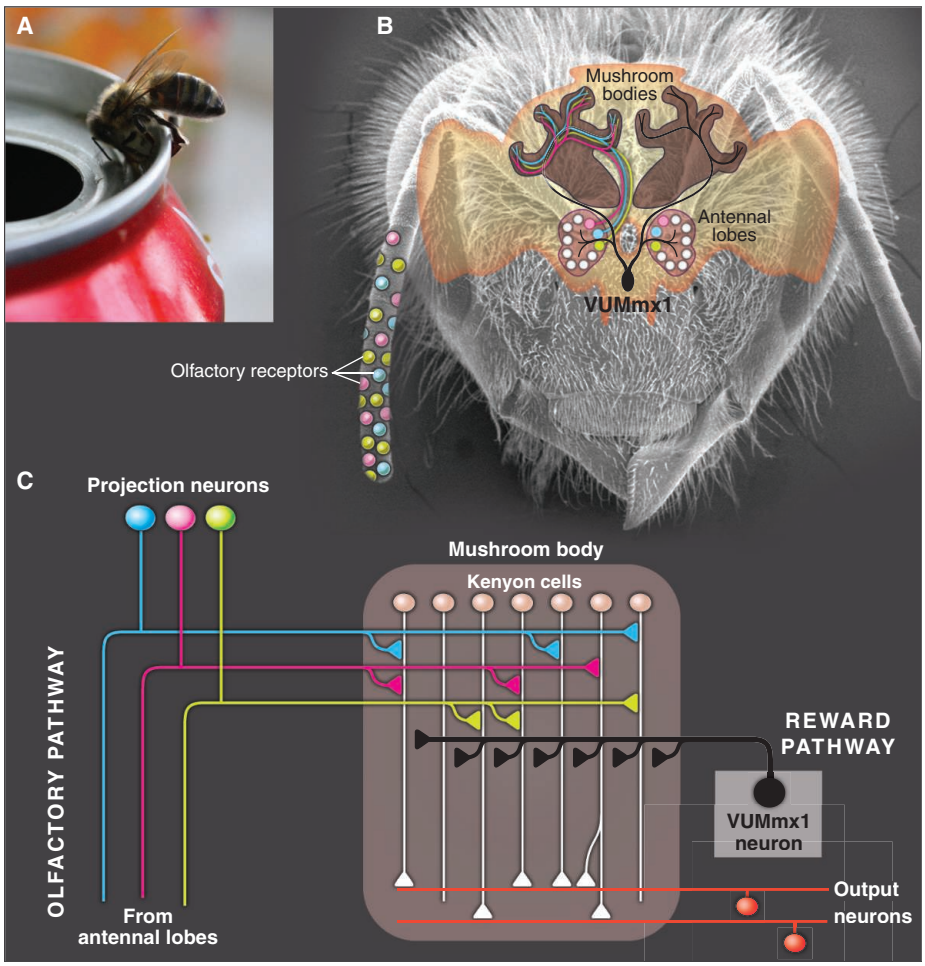
Caffeine Boosts Bees' Memories

Lars Chittka and Fei Peng

Caffeine in floral nectar enhances the memory of bees for the flowers' scent by altering response properties of neurons in the bee brain.

Pollination systems are biological markets, where flower visitors choose between flower species on the basis of their quality, such as the sweetness and amount of nectar per flower. Plants in turn compete for pollinators and advertise their product through colorful visual displays and scents. A key challenge in floral advertising is that signals must be not only attractive but also memorable (*1*): The more distinct a flower signal, the more likely a pollinator is to remember it, increasing the probability that pollinators will visit more flowers of this species while ignoring competing flower species. On page 1202 of this issue, Wright *et al*. (*2*) report that

Psychology Division, School of Biological and Chemical Sciences, Queen Mary University of London, Mile End Road, London E1 4NS, UK. E-mail: l.chittka@qmul.ac.uk



Caffeine junkies of the wild? (**A**) Honeybees often consume caffeinated drinks from discarded cans. Wright *et al*. show that some plants manipulate the memory of bees by adding caffeine to their nectar. (**B**) Various antennal odorant receptor types, each responsive to specific chemicals, send information to the mushroom bodies via the antennal lobes (*12*). A ventral unpaired neuron (VUMmx1) mediating the sucrose reward signal also connects to mushroom bodies (*9*). [Adapted from (*6*)] (**C**) In the odor-learning circuitry in the bee brain, projection neurons connect to Kenyon cells in the mushroom bodies (*12*). As Wright *et al*. show (*2*), simultaneous input to Kenyon cells from olfactory and reward pathways might strengthen synaptic connections between Kenyon cells and output neurons and between projection neurons and Kenyon cells. Caffeine increases transmission at the synapses between projection neurons and Kenyon cells and also enhances Kenyon cell excitability, facilitating the formation of long-term memories for floral scents (*2*).

some plant species appear to gain an unfair advantage in this competitive market by manipulating the memory of bees with psychoactive drugs.

Many plants contain alkaloids such as caffeine and nicotine. Their bitter taste deters herbivores; at high concentrations, they are toxic. The nectars of some flowers, in addition to various sugars, also contain such secondary compounds (*3*). This presents a puzzle, because pollinators typically reject bitter nectar (*4*). However, at low concentrations, bees appear to prefer caffeine-containing nectar (see the figure, panel A) (*3*). Wright *et al*. show that caffeine concentrations in the nectar of various *Coffea* and *Citrus* flower species never exceed levels at which they might deter bees. Hence, flowers are careful not to leak too much bitterness into the sweetness of their nectar. But why is there caffeine in nectar at all?

In mammals, caffeine is a cognitive enhancer (*5*). Wright *et al*. show that caffeine also has a dramatic effect on the long-term memory of bees. The authors trained bees to associate a floral scent with a sucrose reward. If, during training, the reward droplet contained caffeine, twice as many bees remembered the scent 3 days later.

The olfactory receptors of insects are distributed along their antennae (see the figure, panel B). Receptor cell axons extend to the primary olfactory centers, the antennal lobes (*6*). From the antennal lobes, projection neurons connect to the mushroom bodies, which mediate sensory integration and learning in insect brains (*7*). The mushroom bodies of honeybees contain about 370,000 so-called Kenyon cells. The projection neurons appear to release acetylcholine as the primary neurotransmitter, which binds to acetylcholine receptors in Kenyon cell dendrites (*8*). The mushroom bodies also receive information about sugary rewards from the bee's mouthparts by way of the VUMmx1 neuron, the single neuron that constitutes the reward pathway in bee olfactory learning (*9*).

Wright *et al*. found that caffeine increases the excitability of Kenyon cells. Blocking the acetylcholine receptors of Kenyon cells reverses the caffeine effect. This makes it likely that the effects of caffeine are due to increased activity of projection neurons from the antennal lobes.

The authors propose that the increased activation of Kenyon cells could be due to the interaction of caffeine and adenosine receptors in projection neurons. In mammals, adenosine acts as an inhibitory neuromodulator, and caffeine is a known antagonist of adenosine (*5*). The application of DPCPX (an antagonist of adenosine receptors) to bee brains produces a similar excitation of Kenyon cells as caffeine, indicating that the observed effects of caffeine on long-term memory could indeed be via blocking of adenosine receptors (*2*).

It is thus conceivable that caffeine facilitates strengthening of synaptic connections between Kenyon cells and olfactory projection neurons activated by a particular floral scent, especially under the modulatory influence of signals from the reward pathway (see the figure, panel C) (*7*). Connections between Kenyon cells and other neurons further downstream could also be affected (*2*).

The discovery of the cognitive effects of psychoactive drugs in floral nectar opens new perspectives in the competitive race between plant species to lure pollinators. Because these substances occur in many plant tissues (as deterrents to herbivores), they could be added to nectar at little extra cost to the plant, with profound effects on pollinator behavior. If as a result of caffeine ingestion, bees remember the traits of the flowers better, they might be more likely to stay faithful to these flowers and disregard others. Further research may show whether drugs in nectar not only influence pollinator preference via enhanced memory for floral traits but also might have addictive effects. These would be recognizable by drug seeking despite known adverse

effects [such as predation risk at flowers (*10*)], relapses after periods of abstinence, or withdrawal symptoms (*11*).

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MATHEMATICS

Getting the Jump on Explosive Percolation

An analytical approach to explosive dynamical percolation yields general conditions for the transition to be a continuous or discontinuous process.

Robert M. Ziff

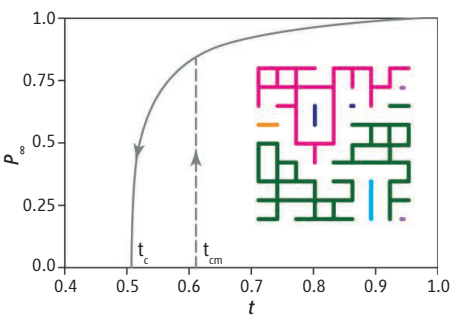
Percolation refers to the formation of long-range connectedness or conductivity in random systems. Simple models for percolation were independently devised in the areas of polymer science (*1*) and mathematics (*2*) in the 1940s and '50s, and have been both a persistent theoretical challenge and an enduring practical paradigm ever since. In the past decade, percolation has become a central problem in probability theory, and has figured in the work of two recent Fields medalists (*3*). A recent and somewhat controversial development concerns looking at the dynamics of percolation under various global bond selection rules and how percolating systems make the transition from being disconnected (or comprising a group of disconnected clusters) to being fully connected. It had been shown that the transition can proceed explosively, in which the transition is discontinuous (*4*), but that scenario was later challenged when it was shown that for some specific systems, such a transition is in fact continuous. On page 1185 of this issue, Cho *et al.* (*5*) show analytically and numerically that the explosive percolation transition can be either continuous or discontinuous, depending on the bias against certain "bridging" bonds and the dimensionality of the system.

In standard percolation, the probability P_∞ that a given point is part of a percolating cluster is a continuous function of the bond occupation probability p, the probability that a bond is conducting or open. P_∞ is zero for cases where $p < p_c$ but rapidly grows for $p > p_c$, where p_c is the percolation threshold that signals the onset of long-range connectivity. In an infinite system and for p slightly greater than p_c, P_∞ behaves as $P_\infty \approx (p - p_c)^\beta$, where β is a fractional power, such as $5/36 \approx 0.139$ in two dimensions (*6*), so the derivative dP_∞/dp is infinite at p_c. Being continuous, the percolation transition can be considered to be a kind of second-order phase transition.

Bonds can be added one at a time and clusters merged as they are added, leading to a dynamical percolation transition. Achlioptas *et al.* (*4*) introduced a new model of percolation in which two candidate unoccupied bonds are simultaneously considered, and only the one that joins the smaller cluster-size sum or product is added. Simulating this model, they found that the percolation transition is delayed to a higher value of p, but then occurs in an explosive and seemingly discontinuous manner, more akin to a first-order phase transition. Similar behavior is observed in many other systems, including regular lattices, when a variety of bond-addition rules are used (*7*–*10*).

Distinguishing a discontinuity from a sharp continuous transition in a finite system can be tricky, however. In 2010, da Costa *et al.* (*11*) showed theoretically that the random-graph model was in fact continuous under a modified product rule, but with a very small β (~0.056), which makes it nearly indistinguishable from a discontinuous transition. More investigation and proofs of the continuity followed for a variety of systems (*12*, *13*), and it is now generally accepted that the original two-choice product rule leads to a continuous transition, but it wasn't previously proven for a Euclidean lattice such as the simple square lattice.

Still, there remain some undoubtedly discontinuous explosive transitions out there. For example, a simple model of joining only the bond that gives the smallest product over all bonds in the system is discontinuous—the last bond joins two huge clusters together. A hierarchical long-range model also shows a discontinuity (*14*).



Joining the dots. Schematic of the probability P_∞ that a point belongs to the spanning cluster versus the occupation probability t, for regular percolation of $m = 1$ (solid line) and for a typical case with $m > 1$ (dashed line). A jump in P_∞ for $m > 1$ is evident. (**Inset**) A typical bond configuration showing a later stage where most of the sites belong to two distinct clusters. The algorithm preferentially puts new bonds along edges that do not connect the two clusters.

Cho *et al.* introduce a model of percolation with a new global constraint on a Euclidean lattice: If a candidate bond causes the system to percolate from one side to another, then the model biases against it. Specifically, m candidate bonds are chosen simultaneously, and if any are nonbridging, then one such bond is chosen randomly and occupied. If they are all bridge bonds, then one of those is chosen and occupied. Equivalently, just one unoccupied bond can be chosen and occupied (if it is a nonbridge bond) or occupied with an appropriate probability (if it is a bridge bond).

Now, $m = 1$ corresponds to standard percolation with critical bond occupancy t_c. For $m > 1$, Cho *et al.* observe that percolation is delayed until a later occupancy $t_m > t_c$, but

Department of Chemical Engineering, University of Michigan, Ann Arbor, MI 48109, USA. E-mail: rziff@umich.edu