of researchers[3,4], but primarily because *Orrorin* is claimed to be about 6 million years old. This makes it 1.5 million years older than *Ardipithecus ramidus*[5], the oldest previously recognized candidate for the earliest hominin. (Hominins include modern humans and fossil species more closely related to them than any other living species.) Significantly, *Orrorin*'s age falls within the molecularly determined range of the last common ancestor between humans and the African apes (8–5 million years ago). The authors also argue that *Orrorin* is on the direct line leading to modern humans, whereas most of the members of the genus *Australopithecus* are not (Fig. 1a). Furthermore, they reposition *Ardipithecus* as an ancestor of the African apes, rather than as the first known human ancestor.

The great age of *Orrorin* does not seem to be in serious question. The geology of the Lukeino Formation is well known[2]; the volcanic tuffs in this formation have been securely dated at 6.2–5.6 million years old by radiometric techniques[6]; and there is little doubt that the specimens come from the Lukeino Formation sediments[2]. It is difficult, though, to have the same confidence in Senut and colleague's conclusions about human evolutionary history. They adopt a simple two-branch evolutionary tree for the hominins (Fig. 1a). One branch leads from *Orrorin* to *Homo* through the novel intermediary genus *Praeanthropus*; the other leads to *Australopithecus* and extinction. This simple phylogeny contrasts starkly with mainstream ideas about human evolution, and glosses over many areas of controversy and uncertainty.

The picture is further complicated by last week's announcement of yet another new hominin genus and species, the 3.5–3.2-million-year-old *Kenyanthropus platyops* from West Turkana in Kenya[7,8]. At least 13 known hominin species from Africa existed before *Homo erectus*, and this period of our evolutionary history now looks more like a tangled bush than a simple tree (Fig. 1b). There are only a few points of consensus among most palaeoanthropologists. One is that there are four hominin genera (*Ardipithecus, Australopithecus, Paranthropus* and *Homo*), with the new *Kenyanthropus* making five. Another is that the big-toothed and massive-jawed genus *Paranthropus* (*P. aethiopicus, P. robustus* and *P. boisei*) represents a dead-end branch of the bush.

To understand Senut and colleagues' interpretation of *Orrorin* it is necessary to appreciate their reasons for creating an additional hominin genus, *Praeanthropus*. Senut has long believed that the skeleton, and not the skull and teeth, is the best guide to hominin evolutionary relationships[9]. She argues that the skeletal evidence suggests a very old division in hominin locomotor ability. One lineage, characterized by climbing and bent-legged bipedal walking, led to most of the members of the genus *Australopithecus* (including *Paranthropus*); the other lineage, comprising straight-legged walkers, led from other members of the genus *Australopithecus* through *Praeanthropus* and *Homo rudolfensis* (now *Kenyanthropus rudolfensis*[7]) to *Homo sapiens*. The genus *Praeanthropus* represents those members of the genus *Australopithecus* that Senut interprets as having a skeleton suggesting more modern walking (*A. anamensis*, and some fossils normally included in the species *A. afarensis*)[9]. She also suggests that this phylogeny is supported by evidence from the teeth and jaws.

Most palaeoanthropologists do not recognize a major dichotomy in hominin locomotor ability before the evolution of *Homo ergaster*, around 1.9 million years ago, and recent analyses of the *A. anamensis* skeleton suggest that it was much like that of other members of the genus *Australopithecus*[10,11]. Senut's claim for more modern walking for *Orrorin*, linking it with *Praeanthropus* and *Homo*, is based on detailed aspects of the anatomy of the upper part of the thigh-bone that are open to alternative explanations. For example, she and her colleagues argue that the head of the thigh-bone is very large and human-like in relation to the size of the neck of the bone. This is true, but it is also the case that *Orrorin* is no more similar to *Praeanthropus* in this feature than it is to *Australopithecus*, which also falls within the human range of human variation.

Senut and colleagues claim[1] that *Orrorin*'s relatively small, thick-enamelled molars support their interpretation that *Orrorin* is a direct ancestor of modern humans to the exclusion of *Ardipithecus* and most members of the genus *Australopithecus*. But tooth size and enamel thickness correlate with diet[12]: in the absence of other compelling evidence to link *Ardipithecus* with the African apes, or *Orrorin* with humans, it is premature to make such bold claims.

The age of *Orrorin* undoubtedly makes it a highly important addition to the debate about human origins. But we are a long way from a consensus on its role in human evolution. There are many alternative hypotheses that are equally defensible, including some in which *Orrorin* is not a hominin. *Kenyanthropus* and other new, and as yet little known, hominins such as *Australopithecus garhi*[13] introduce further uncertainty. It also appears that cranial and dental anatomy does not necessarily mirror molecularly determined phylogenies in modern primates[14], which casts considerable uncertainty on anatomically based evolutionary trees. For now, at least, it is probably best to avoid naming ancestors, and maintain a simple division: that between hominins of archaic aspect (*Orrorin*, *Ardipithecus*, *Australopithecus* — including *Paranthropus* — and *Kenyanthropus*) and hominins of modern aspect (*Homo sapiens* and the remaining species of *Homo*)[15,16]. ■

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Neurobiology

# Cannabinoids act backwards

MacDonald J. Christie and Christopher W. Vaughan

Cannabis is useful for treating many ailments, but has unwanted side effects. Drugs that control signalling by cannabinoids found naturally in the body might be more useful.

Preparations from the plant *Cannabis sativa* have been used since antiquity, not only for their intoxicating effects, but also to treat a number of ailments[1,2]. The main active component of these preparations, $\Delta^9$-tetrahydrocannabinol, produces most of its effects on the central nervous system by interacting with specific cannabinoid receptors on nerve cells. Under normal circumstances, these receptors are thought to be one element of a neurotransmitter system that controls neuronal excitability. Other components of this putative signalling system include cannabinoids that are found naturally in the body, as well as cellular mechanisms by which these 'endocannabinoids' are synthesized, transported and metabolized[3]. But it has not been clear how important this system really is, because of a lack of direct evidence for the synthesis,

release and effects of endocannabinoids at the junctions between nerve cells (synapses) under natural conditions.

Writing on page 588 of this issue[4], Wilson and Nicoll provide an answer. At synapses, one neuron (the presynaptic one) releases neurotransmitter molecules that diffuse to another (postsynaptic) neuron, either inhibiting or stimulating it. Wilson and Nicoll show that endocannabinoids can be formed in single postsynaptic neurons in response to physiologically relevant stimuli, and that the endocannabinoids diffuse back from the stimulated cell to act on receptors on a presynaptic neuron. This has the effect of decreasing inhibitory inputs to the postsynaptic cell. The study also highlights the importance of endocannabinoid transporters in controlling this process. Meanwhile, Ohno-Shosaku and colleagues[5] and Kreitzer and Regehr[6], writing in *Neuron*, show that such 'retrograde' endocannabinoid signalling is a general phenomenon, occurring at certain excitatory and inhibitory synapses in the hippocampal region of the brain, and at some excitatory synapses in the cerebellum.

It was already known that strong depolarization (excitation) of so-called pyramidal neurons in the hippocampus suppresses inhibitory inputs to pyramidal neurons from presynaptic cells. This phenomenon, known as depolarization-induced suppression of inhibition (DSI)[7], requires an influx of calcium ions into the postsynaptic neuron. It was also known that the synthesis of endocannabinoids in neurons is stimulated by an increase in calcium concentration, and that the most common type of cannabinoid receptors, called CB1 receptors, are expressed on inhibitory presynaptic nerve terminals that form synapses with pyramidal neurons.

The three groups of authors[4–6] have integrated these findings and implicated an endocannabinoid acting on the CB1 receptor as the retrograde messenger that is released from a depolarized postsynaptic neuron to produce DSI (Fig. 1). They established this by using synthetic molecules that antagonize the CB1 receptor or mimic the effects of endocannabinoids. They also used a synthetic molecule that binds to and activates the CB1 receptor and so prevents further activation of the receptor by endocannabinoids. Furthermore, Wilson and Nicoll[4] and Ohno-Shosaku *et al.*[5] find that, in response to depolarization or induction of action potentials in single hippocampal pyramidal neurons, increases in calcium concentration occur that are sufficient to trigger the production of endocannabinoids, which act as retrograde messengers. These studies have incidentally resolved another controversy by showing that another candidate neurotransmitter, glutamate, is not involved in DSI.

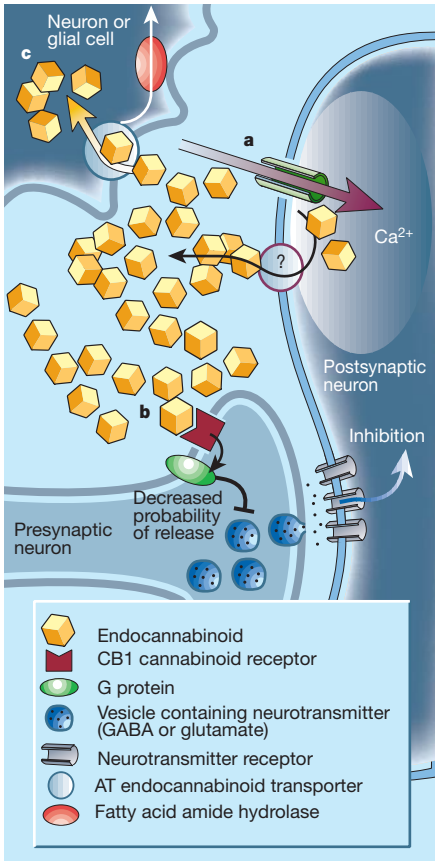


Figure 1 **Cannabinoids found naturally in the body (endocannabinoids) act as retrograde messengers within the brain. The model shown here is based on three new papers[4–6].** a, **Excitation of a neuron causes its depolarization and an influx of calcium ions. This stimulates various phospholipases, which start the synthesis of endocannabinoids, such as anandamide and 2-arachidonylglycerol. These are released from the neuron by an unknown mechanism (?).** b, **Endocannabinoids freely diffuse away to bind to CB1-type cannabinoid receptors on the presynaptic terminals of neurons that form synapses with the stimulated neuron. This reduces the probability of inhibitory neurotransmitters being released. The effects of the endocannabinoids are mimicked by the active component of cannabis, $\Delta^9$-tetrahydrocannabinol, as well as by synthetic agonists, and are blocked by antagonists of the CB1 receptor (not shown).** c, **Endocannabinoids are taken up into neuronal and glial cells by a transporter and then broken down, possibly by the membrane-bound fatty acid amide hydrolase. So the amount of endocannabinoid available to regulate presynaptic CB1 receptors is regulated by uptake and degradation.**

The endocannabinoids that produce these retrograde signals have not yet been identified. But Wilson and Nicoll have a possible answer. They show that a transporter protein called AT that is found on nearby neurons or on glial (non-neuronal) cells, and which mops up the endocannabinoids anandamide and 2-arachidonylglycerol from the synapse, is likely to be important in retrograde signalling. The authors found that AM404 — a drug that inhibits this transporter — both mimics and partially prevents DSI. How could this come about?

Presumably, by inhibiting the transporter, the drug initially prevents the endocannabinoids from being removed from the synapse, and so enables them to activate CB1 receptors. The lingering endocannabinoids acting on the CB1 receptor might then prevent further activation of the receptor by endocannabinoids produced by DSI. The significance of this endocannabinoid transporter was previously unclear, because the known endocannabinoids are lipophilic, and so were thought to diffuse freely across neuronal and glial membranes. But Wilson and Nicoll now show that the transporter is physiologically important for ending endocannabinoid signals.

These results also suggest that anandamide and 2-arachidonylglycerol are the key endocannabinoids in retrograde signalling, but this is by no means certain. Experimentally manipulating enzymes that might be involved in the synthesis and breakdown of endocannabinoids — for example, by using inhibitors of the enzyme fatty acid amide hydrolase — may help to narrow the range of candidate endocannabinoids. Moreover, the mechanism by which endocannabinoids are exported from a stimulated neuron needs to be determined. It is also unknown whether endocannabinoids can be generated physiologically using stimuli other than depolarization-induced calcium entry. For example, perhaps their production might be activated by other neurotransmitter receptors that are coupled to signalling molecules called $G_q$ and $G_{11}$ proteins, and hence to phospholipase enzymes.

Wilson and Nicoll[4] have also used the parallel alignment of hippocampal pyramidal neurons and their primary dendrites (extensions) to estimate the range of diffusion of the endocannabinoids released from a single neuron in a brain slice. They found that the retrograde signal affects synapses within a radius of about 20 micrometres from the stimulated neuron. But the effects of inhibiting endocannabinoid transport or metabolism on this range have yet to be studied. Such inhibitors might increase the range or intensity of retrograde endocannabinoid signalling at this or other synapses. If, as suggested by Wilson and Nicoll, DSI enhances learning in the hippocampus, then these inhibitors might intensify the process. By contrast, drugs such as $\Delta^9$-tetrahydrocannabinol, which act

directly on all cannabinoid receptors in the hippocampus, have disruptive effects.

Natural and synthetic cannabinoids relieve nausea and vomiting, and stimulate appetite. They also have a range of effects that suggest they might be useful in treating pain, migraine, muscle spasms associated with multiple sclerosis, and glaucoma[1,2]. But cannabinoids also have many unwanted side effects. Our ever-increasing understanding of endocannabinoid signalling has raised hopes that useful drugs without these side effects could be developed[1,2].

Unfortunately, the CB1 receptor is widely distributed throughout the brain and accounts for almost all of the effects of cannabis on memory, cognition, coordination, mood, pain sensation, appetite and sleep. (The only other cannabinoid receptor identified so far, the CB2 receptor, is expressed largely in immune tissues.) So it is unlikely that synthetic drugs that activate the CB1 receptor could sidestep all the unwanted psychotropic effects of cannabis. But drugs that inhibit endocannabinoid transporters would work only to enhance the actions of naturally released endocannabinoids, so might be much more useful. ■

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Plant biology

# Night moves of pregnant moths

Clarence A. Ryan

Tobacco plants attacked by caterpillars release different blends of volatile compounds by day and night. Those released at night tell nocturnal moths not to approach — a signal that benefits both plants and moths.

Volatile chemicals are the language of plants. Through the smell of fresh blossoms, good coffee or a fine wine, their message to humans can be attractive. But plants do not expend valuable energy making these chemicals simply to please humans, and most volatiles have more serious functions. Some, for instance, are important in communicating information to particular insects that is crucial to the survival of the plants, and often the insects as well.

On page 577 of this issue[1], De Moraes and colleagues describe a previously unknown chemical conversation between plants and herbivorous insects (Fig. 1). At night, tobacco plants that are being attacked by caterpillars emit a specific blend of volatile chemicals. Nocturnal moths interpret these chemicals as a signal that they will not be welcome to lay their eggs there. But it isn't just the plant that benefits from these night-time emissions. As the plant is making nasty chemicals to ward off the caterpillars, and may be summoning help from predatory insects, it is advantageous for the moths to keep away.

It is well known that, when being grazed on by herbivorous insect larvae, plants release volatile chemicals such as terpenoids to attract carnivorous insects that will prey on the larvae. For instance, lima-bean plants infested by spider mites release volatile chemicals that attract predatory mites, which in turn feed on the spider mites[2]. The chemicals produced are specific to the type of damage: neither leaves from uninfected plants nor leaves damaged mechanically in the absence of mites release these particular chemicals, so they do not attract the predatory mites. Similarly, corn seedlings and cotton plants damaged by caterpillars release volatile signals that attract parasitic wasps, which lay their eggs in the caterpillars[3]. When the eggs hatch, the larvae dine on their hosts, eventually killing them. These clever ruses contribute to the survival of both the plants and the predators, which do not eat leafy plants. More than 15 plant species, 10 herbivorous insect species and over 10 predatory insect species are known to be involved in such interactions[4].

The process of attracting predatory insects involves the interaction of specific blends of plant volatiles with highly sensitive receptor molecules of the predators. And, taking a step back, the grazing herbivores themselves release chemicals in their saliva that lead to the synthesis and release of the plant volatiles.

For example, a chemical signal from the saliva of beet armyworm caterpillars has been isolated and characterized as *N*-(17-hydroxylinolenoyl)-L-glutamine, also called volicitin[5]. And the saliva of spider mites contains an enzyme, β-glucosidase, that induces volatile emissions from lima-bean plants[6]. But it is not yet known how these 'elicitor' molecules signal to the plants, or how they interact with or complement the plants' own elicitors. Moreover, when the corn and lima-bean plants are attacked these volatiles are even released from undamaged leaves, so presumably a compound from the wound site is transported in the vascular systems of the plant, causing the emission of volatile chemicals from all parts of the plant[4]. Such systemic signalling in response to herbivores is well established[7]. But, except for the polypeptide defence signal called systemin[8], little is known of the chemical nature of the systemic signals produced in plants by insect attacks.

Many herbivorous insect larvae that have large effects on agriculture are hatched from eggs deposited on the plants at night by nocturnal moths. The larvae feed mainly during daylight hours, which is when the plants release volatiles that attract predatory and parasitic insects. Little is known about volatiles that might be emitted at night.

De Moraes *et al.*[1] now show that tobacco plants under attack from caterpillars produce volatiles at night, as well as by day, and that the nocturnal chemicals are different to the daytime ones. The night emissions,

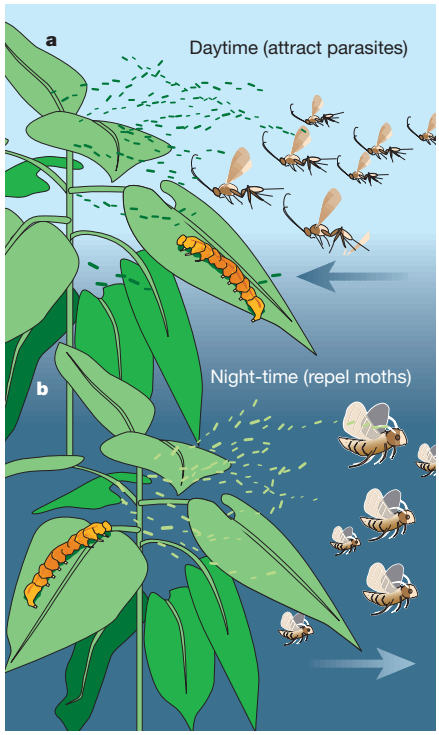


Figure 1 **Tobacco plants use different blends of volatile compounds by day and by night, as shown by De Moraes and colleagues[1].** a, **In daylight, plants under attack by herbivores emit blends that attract parasitic or predatory insects, which destroy the herbivores.** b, **By night, attacked tobacco plants release volatiles that repel nocturnal pregnant moths, which are looking for somewhere to deposit their eggs. Both responses are beneficial to the plants, and also to the insect species involved.**