OLFACTION

Intimate neuronal whispers

It's a touching story of cohabitation and meaningful communication. Two neighbouring fruitfly neurons talk to each other not by means of synaptic junctions but by interactions through the surrounding electrical field. SEE ARTICLE P.66

KAZUMICHI SHIMIZU & MARK STOPFER

To process information, neurons almost always work as discrete, insulated building blocks of the nervous system — they interact only through specialized gateway structures known as synapses, but otherwise keep their cogitations to themselves. This view has been prevalent since Ramón y Cajal and other biologists formulated the 'neuron doctrine' more than a century ago. But on page 66 of this issue, Su *et al.*[1] describe a notable finding: multiple olfactory receptor neurons can directly influence one another non-synaptically, with their combined output forming a description of the olfactory environment*.

Su and colleagues observed this in the fruitfly *Drosophila*, an insect that offers experimenters a terrific toolkit for genetic manipulation. *Drosophila*'s olfactory receptor neurons (ORNs), like those of many animals, are packed into narrow, fluid-filled structures called sensilla. Most sensilla contain more than one ORN. The dendritic projections of these cells touch each other but the neurons themselves are not connected by synapses[2].

The authors focused on a sensillum called ab3, which contains two ORNs (ab3A and ab3B). They exposed the insects to two odorants — methyl hexanoate and 2-heptanone — each of which activates only one of these ORNs. While one odorant was presented continuously in the background, the second odorant was briefly pulsed. As expected, when methyl hexanoate was presented in the background, ab3A began to respond by firing a series of action potentials (spiking). When 2-heptanone was pulsed atop this background, ab3B responded by spiking. Intriguingly, however, as soon as ab3B began to fire, the ongoing response of ab3A suddenly and dramatically decreased (Fig. 1a–c).

To test whether spiking in ab3B was directly responsible for the diminished response of ab3A, Su *et al.* used a genetic trick — they destroyed ab3B to prevent it from firing, and then repeated the experiment. This time, pulsing 2-heptanone had no effect on the firing of ab3A. Through further complementary experiments in ab3, and experiments in other types of sensillum, the researchers showed that spiking in one ORN can inhibit the responses of other ORNs in the same sensillum.

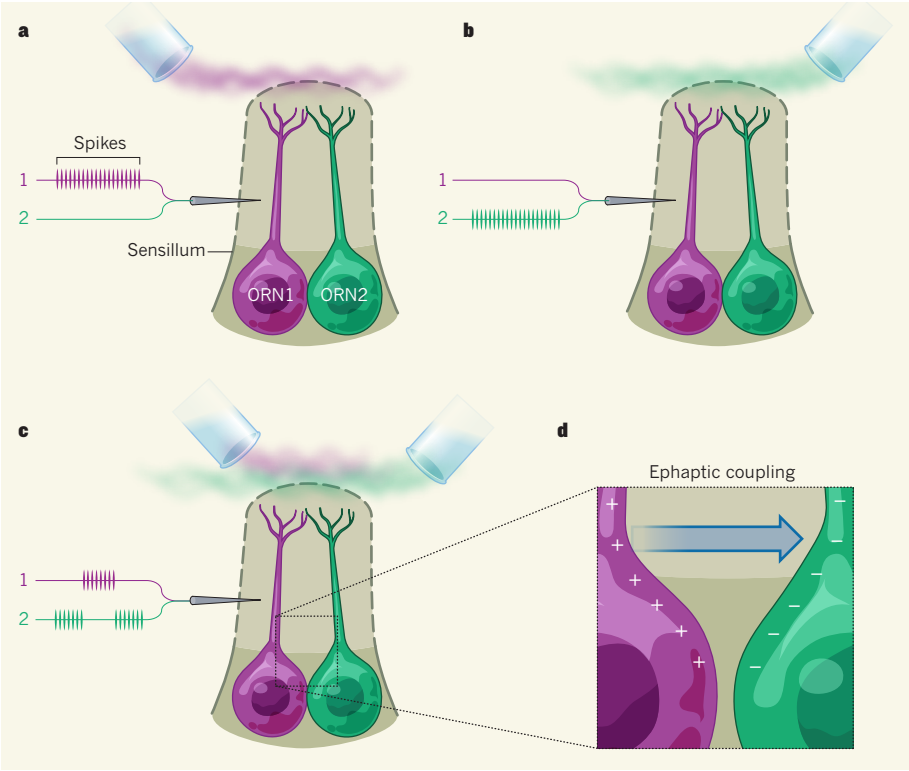

Figure 1 | Close neuronal couples. **a**,**b**, In this simplified example, olfactory receptor neurons 1 and 2 (ORN1 and ORN2) reside within the same sensillum, but do not make synaptic connections. From the spiking responses of these neurons, displayed separately on lines 1 and 2, ORN1 responds to the 'purple' odour (**a**), whereas ORN2 responds to the 'green' odour (**b**). **c**, When a pulse of the purple odour is briefly mixed into the ongoing background green odour, ORN1 transiently responds, and during this time ORN2 is inhibited. **d**, Su *et al.*[1] propose that this inhibition is mediated by a direct electrical field interaction between such closely apposed ORNs through the process of ephaptic coupling.

Is this coupling effect powerful enough to influence the animal's behaviour? To test this, the authors studied another sensillum containing two ORNs, one of which mediates attraction to apple cider vinegar (ACV), and the other repulsion from carbon dioxide. Given a choice between a CO_2–ACV mixture and a CO_2–water-vapour mixture, flies typically prefer CO_2–ACV. Su and co-workers reasoned that this preference could be explained by a strong attraction to ACV; by repulsion from CO_2 that is diminished by the presence of ACV; or by a combination of both. To determine which of these possibilities was true, they used genetic manipulations that allowed the ACV-responsive ORNs to fire action potentials, but blocked them from synaptically activating any 'follower' neurons.

Flies manipulated in this way showed reduced attraction to the CO_2–ACV mixture, but still preferred it to the CO_2–water-vapour blend. These insects could not have been directly attracted to ACV because the synaptic pathway mediating this attraction had been blocked. Moreover, a genetic manipulation that prevents ACV receptors from

*This article and the paper under discussion[1] were published online on 21 November 2012.

responding but leaves CO_2 receptors intact reduced the fly's preference for CO_2–ACV over CO_2–water vapour. Together, these clever and elegant experiments suggest that the flies' behavioural preference for CO_2–ACV can be explained by two factors: attraction to ACV; and reduced repulsion from CO_2, mediated by ACV-receptor-driven inhibition of spiking in the CO_2-responsive neuron.

The authors' additional experiments showed that this receptor-driven inhibition is not mediated through synapses. Instead, they propose that the ORNs interact directly through the extracellular fluid surrounding the neurons by a mechanism called ephaptic coupling[3] (Fig. 1d). When a neuron fires an action potential, electrical charges transiently flow into and out of its membrane. Usually, the impact of this current flow is diffuse and slight outside the neuron. But when the extracellular space is unusually tight and compartmentalized, the impact can, in theory, be strong enough to affect the electrical activity of neighbouring neurons, leading to ephaptic interactions[4].

Ephaptic transmission is particularly interesting in the context of the chemical senses because chemosensory receptors commonly cohabit. In insects, for instance, gustatory receptor neurons — like ORNs — are packed tightly together into sensilla[5]; and in vertebrates, gustatory receptors are intertwined within compact taste buds[6]. Thus, Su and colleagues' report calls for a systematic revision of long-standing views of the first stage of chemosensory processing. On the basis of their data, the receptors seem to function not as fully independent information channels, but rather as interactive components, with the sensillum serving as the autonomous building block of sensory transduction.

What benefits might this arrangement provide for coding sensory information? According to Su *et al.*, it enables the novelty of a stimulus to be detected and encoded right there in the periphery, when new, transient responses suppress ongoing activity of the neuron sensitive to the stimulus. Although little is known of the logic behind the distribution of different types of ORN into sensilla, the process of making behavioural choices might often begin as interactive antagonisms between strategically paired, contiguous ORNs. Moreover, the timing of spiking in ORNs may be shaped in part by ORN–ORN interactions; this timing underlies the more elaborately patterned neural odour codes arising downstream[7]. More work is needed to determine the mechanistic details and behavioural consequences in this fascinating story. ■

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ORGANIC CHEMISTRY

Toolkit of reagents to aid drug discovery

Reagents have been developed that allow carbon–hydrogen bonds on benzene-like compounds called heterocycles to be converted directly into carbon–carbon bonds. The finding will be a boon to medicinal chemists. SEE LETTER P.95

WILLIAM J. PITTS

When designing medicines, the difference between success and failure can hinge on small variations made to the molecular structures of candidate drugs during the optimization phase of drug discovery. In this issue, Fujiwara *et al.*[1] report the development of a toolkit of reagents that can be used to attach a variety of chemical groups to heterocycles — benzene-like rings containing atoms such as nitrogen, as well as carbon, that are frequently found in drug molecules. The toolkit should make it quicker to generate analogues of candidate drugs for biological testing, and improve the metabolic stability of compounds containing heterocycles. It also provides medicinal chemists with a powerful strategy for making 'substituted' heterocycles — heterocyclic compounds to which chemical groups are attached — and so offers a tactical advantage for the optimization phase of drug discovery*.

*This article and the paper under discussion[1] were published online on 28 November 2012.

Fujiwara and colleagues' toolkit consists of ten zinc bis(alkanesulphinate) salts. These reagents, which have practical uses, are precursors to alkyl radicals — species known to react with a wide variety of heterocycles, including those contained in drugs and natural products. The authors found that their zinc salts typically react at carbon–hydrogen (C–H) bonds adjacent to nitrogen atoms in heterocycles, creating a carbon–carbon (C–C) bond to the alkyl group of the salt (Fig. 1). The resulting products are not commercially available and can be difficult to prepare using traditional synthetic approaches, requiring many steps to complete.

The reactions do not require 'pre-functionalized' sites in heterocycles — that is, there is no need to incorporate special groups at the reaction sites to allow C–C bond formation to occur. This makes it easier to synthesize substrates for the reactions, and means that substrates can be made from simple (and therefore often cheap) starting materials. What's more, Fujiwara *et al.* report that the reactions do not affect potentially reactive sites or groups that might be present elsewhere in the starting material. This means that structurally complex compounds obtained as intermediates in the late stages of a synthetic pathway can be used as substrates for the reactions, making it easier to prepare several analogues of a compound from a common intermediate. It also means that there is no need for protection strategies, in which reactive sites are temporarily modified to stop them taking part in unwanted side reactions, reducing waste and expense.

Fujiwara *et al.* observed that when two different zinc bis(alkanesulphinate) salts were added sequentially to the same heterocycle, new C–C bonds formed specifically at two different sites in the substrate. This ability to perform sequential reactions further increases the complexity of the substituted heterocycles that can be produced using the toolkit. The researchers also report that the reactions can proceed under 'mild' conditions that are unlikely to cause substrates to decompose; that they tolerate several chemical groups known to be especially prone to side reactions; and that they can be performed in organic solvents, in water, and even in the isolated fluid contents of cells. Fujiwara and colleagues went on to demonstrate that the reactions proceed in buffer solution in the presence of an enzyme, β-lactamase, without adversely affecting the enzyme's activity. This result leads them to suggest that their chemical toolkit can facilitate the attachment of drugs to proteins such as antibodies, which can increase the therapeutic effect of the drug substance and target delivery to specific cells.

Several of the alkyl groups in the zinc bis(alkanesulphinate) salts contain fluorine atoms. This makes them particularly useful to medicinal chemists, who are increasingly