and methotrexate into cells. This result was unsurprising, given that SLC19A1 has previously been linked[3] to sensitivity to methotrexate, but it confirmed the power of the authors' approach.

Kanarek and colleagues found that decreasing the expression of a gene that encodes the enzyme formimidoyltransferase cyclodeaminase (FTCD) also reduced the sensitivity of the cancer cells to methotrexate. FTCD degrades histidine, an amino acid that humans must obtain from their diet because the body cannot synthesize it. The authors reasoned that, because FTCD requires THF as a cofactor for its activity, a decrease in the intracellular levels of FTCD could help to limit the reduction of cellular pools of THF that occurs with methotrexate treatment. Consistent with this hypothesis, the authors found that when the levels of FTCD were reduced by gene editing, THF depletion in human cancer cells treated with methotrexate was less than that seen in methotrexate-treated cells in which FTCD levels were not reduced.

On the basis of these findings, the authors predicted that the manipulation of other steps in the histidine-degradation pathway could affect the efficacy of methotrexate treatment. To test this, they again used gene editing to decrease the expression of other genes in this pathway in human cancer cells grown *in vitro*. They found that such decreases did indeed mimic the effect of decreased FTCD levels in preserving the cellular pool of THF in methotrexate-treated cells.

The authors then analysed a panel of cancer cell lines, and observed a correlation between cell lines that had low expression of the enzyme histidine ammonia lyase (HAL), which acts at the first step in the histidine-degradation pathway, and low sensitivity to the effects of methotrexate. Moreover, when Kanarek and colleagues analysed data[4] available for people who have a form of leukaemia that is commonly treated with methotrexate, they found that patients who had high levels of HAL expression had higher survival rates than those with low HAL expression levels. Perhaps this is because methotrexate is more effective when high levels of HAL boost histidine degradation and the depletion of THF pools by FTCD.

The authors therefore proposed that greater cellular histidine degradation should deplete the THF pool and thus increase the effectiveness of methotrexate. To explore this prediction, they generated tumours in mice, and gave the animals methotrexate and a histidine-rich diet. They treated the animals with lower doses of methotrexate than those normally used in such models, to assess whether histidine had a synergistic effect in this context. The histidine-rich diet did indeed boost tumour-growth inhibition in response to low-dose methotrexate compared with the effects of methotrexate in animals that did not receive a dietary histidine boost.

An unexpected and exciting finding of Kanarek and colleagues' work is that, in their mouse models, the toxicity that is usually seen in non-cancerous tissues with methotrexate treatment did not occur when the animals were given a histidine-rich diet, despite the extra toxicity that would have been expected as a result of the depletion of THF in these tissues. The results not only indicate that histidine can increase methotrexate-driven cytotoxicity in cancer cells, but also raise the possibility that some mechanism is reducing the toxic effects of THF depletion in normal tissues. Do normal and tumour tissue differ in sensitivity in this context as a result of having different thresholds for toxicity, or is the rate of histidine degradation different in normal and tumour tissues?

Given the technical challenges involved in measuring certain intermediate molecules in the histidine-degradation pathway, histidine's contribution to the folate pool still needs to be quantified *in vitro* and *in vivo* to validate the authors' model. Furthermore, there are other pathways[5], in addition to that of histidine degradation, for example degradation of the amino acid methionine, that can affect the THF level in cells. It will be important to investigate the extent to which such pathways contribute to the THF pool, and whether they also affect methotrexate-associated toxicity.

Kanarek and colleagues' work indicates the potential value of conducting clinical trials to investigate whether histidine supplementation can boost the effectiveness of methotrexate in cancer treatment. If this is the case, further clinical trials would be needed to determine the optimal dosage. Histidine supplementation might provide an inexpensive and safe strategy for increasing the efficacy of methotrexate. Supplementation with low doses does not have adverse effects in humans, although doses of more than 8 grams of histidine a day can lead to zinc deficiency[6]. However, there have been no extensive studies to investigate possible detrimental effects of a histidine-rich diet in humans.

Finally, these results might have relevance for people with rheumatoid arthritis or other chronic autoimmune diseases, in which low-dose methotrexate is the standard treatment[7] and folate supplements are the only strategy currently available to limit side effects. Could a histidine-rich diet reduce side effects in these people also, and increase their well-being? ■

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NEUROBIOLOGY

How the head governs the heart of fly species

The males of two closely related species of fly respond differently to a female pheromone. It emerges that this difference is due to alterations in the activity of an evolutionarily conserved neural circuit in the brain. SEE ARTICLE P.564

NICOLAS GOMPEL
& BENJAMIN PRUD'HOMME

Particular sensory signals — a certain odour or taste, for instance — can trigger opposing responses in different species. What changes in neural circuits could elicit such contradictory behaviours? On page 564, Seeholzer *et al.*[1] compare the circuits that control courtship in males in two closely related species of fruit fly that behave differently when exposed to a particular pheromone. They find an explanation for these opposing behaviours that few neuroscientists would have predicted.

While wandering on a rotten apple, a male fruit fly is likely to encounter a variety of other flies, only a few of which will be females of the same species. Identifying and courting these conspecific females is a crucial step in securing the male fly's reproductive success. But this is not always an easy task. For instance, two sister species of fruit fly — *Drosophila melanogaster* and *Drosophila simulans* — are often found on the same fruits. Males of these species therefore rely heavily on sensing pheromones to identify conspecific females[2].

To test whether a female fly is of the same species, the male touches her abdomen with

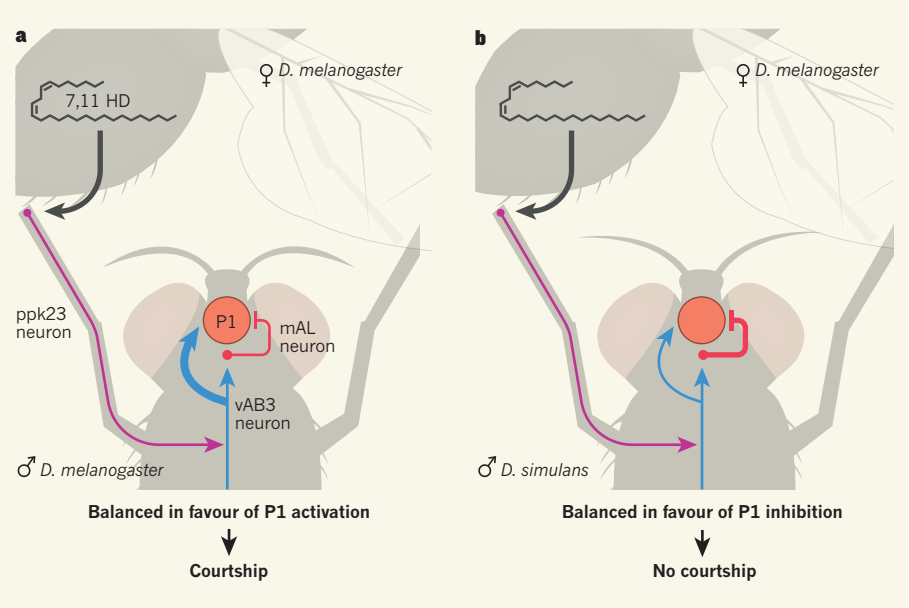

Figure 1 | Changes in the activity of a courtship-promoting neural circuit. **a**, Female fruit flies of the species *Drosophila melanogaster* produce the pheromone 7,11-HD. Seeholzer *et al.*[1] report that *D. melanogaster* males identify females of their species through neurons in the foreleg that express the protein Ppk23. These ppk23 neurons activate a population of neurons called vAB3 that, in turn, activates neurons in the P1 brain region, promoting courtship behaviour. vAB3 neurons also activate the mAL neuron population, which inhibits P1. In this species, vAB3 activation overrides mAL inhibition to trigger P1 activity, leading to courtship. **b**, The authors find that this neural circuit is evolutionarily conserved in males of a sister species, *Drosophila simulans*. However, mAL-mediated inhibition overrides vAB3 activation, so that P1 is inhibited and courtship behaviours are aborted. Whether this difference reflects changes in the activity of mAL neurons or changes in how the inputs are detected by P1 neurons remains unclear.

his foreleg, sampling her pheromones using sensory neurons. *D. melanogaster* females produce the pheromone 7,11-HD, which promotes courtship behaviour when sampled by *D. melanogaster* males[2]. By contrast, *D. simulans* females do not produce 7,11-HD, and *D. simulans* males react to the pheromone (on either *D. melanogaster* females or *D. simulans* females doused with 7,11-HD) by aborting courtship behaviour. Hence, 7,11-HD is detected by males from both species, but elicits contrary behavioural responses in each.

What differences in the nervous systems of the male flies could account for the opposing role of 7,11-HD in regulating courtship decisions in these two species? Seeholzer *et al.* set out to answer this question by comparing the architecture and function of the neuronal circuits that govern courtship behaviour in both. To achieve this, the authors used a combination of sophisticated techniques: the genetic manipulation of specific populations of neurons; the activation of particular neurons with light using a technique called optogenetics; and the observation of neuronal activity in real time using functional imaging.

In *D. melanogaster* males, the decision to initiate courtship is made by the activation of a region in the brain called P1, which contains neurons that integrate multiple sensory signals from outside the brain[3]. Seeholzer *et al.* found that P1 has the same role in *D. simulans*. Moreover, the neural pathway that detects 7,11-HD and propagates the sensory input to P1 is structurally similar in *D. melanogaster* and *D. simulans* (Fig. 1).

The authors showed that 7,11-HD is detected by a few taste-perceiving neurons in the male foreleg of each species that are characterized by expression of the protein Ppk23. These neurons activate a population of neurons called vAB3 that, in turn, directly excites P1 neurons[4]. However, the researchers demonstrated that vAB3 neurons also activate a population of neurons called mAL that directly inhibits P1 neurons[4]. The pheromone-processing pathway therefore bifurcates into excitatory and inhibitory routes that converge on P1. This circuit presumably provides a mechanism for setting pheromone responses and for tightly controlling the excitation of P1.

"The authors have shown clearly that evolutionary changes can be embedded more deeply in brain circuits."

If the structure of the pheromone-processing pathway is evolutionarily conserved between species, how does 7,11-HD elicit a net excitation of P1 to trigger courtship in *D. melanogaster*, but not in *D. simulans*? Seeholzer *et al.* found that it's simply a matter of balance. The mAL-mediated inhibition of P1 neurons completely masks their excitation by vAB3 neurons in *D. simulans*, resulting in a net inhibition of P1. But mAL activity only dampens the activation of P1 by vAB3 in *D. melanogaster*.

Seeholzer and colleagues' work therefore suggests that it is not the architecture of the circuitry that has changed during the evolution of the two species, but the relative weight of the inhibitory and excitatory pathways that converge on P1 neurons. This is unexpected, because the idea that changes outside the brain can explain behavioural diversity has long dominated this field[5–10]. But the authors have shown clearly that evolutionary changes can be embedded more deeply in brain circuits, where no one has looked so far. This is a provocative new conceptual framework that explains how a behaviour can change between closely related species.

The most interesting aspect of Seeholzer and colleague's work is undoubtedly the unexpected nature of the findings. But the study's combination of state-of-the art techniques also makes it a technical triumph, for two reasons. First, using neurogenetic tools designed for the model organism *D. melanogaster* in the non-model species *D. simulans* was a challenge. Second, comparing the structure and function of individual circuit components between species, in the haystack of neurons that makes up the brain, is uncharted territory.

Of course, several questions remain. For instance, what caused the change in balance between the inhibitory and excitatory branches of the courtship pathway? Perhaps it was a progressive change that emerged from genetic variation between individuals belonging to the last common ancestor of these species. Under sexual selection, initial variations that caused two groups of males to respond differently to 7,11-HD, as well as two groups of females to produce different levels of the pheromone, might have gradually built a barrier between the incipient species. The genetic basis of the difference in neural-circuit activity between the two species, however, remains to be defined.

It is also unclear how fine-tuning of the circuit differs between the species. Seeholzer *et al.* showed that vAB3 and mAL neurons are active in both species, so there must be a difference in the way their signalling is propagated to P1. Perhaps there are changes in the levels of signalling by mAL neurons, or maybe P1 neurons respond differently to inhibitory inputs in each species.

Finally, the study highlights that integrative nodes in neural circuits such as P1 might be poised to accommodate evolutionary changes that underlie variations in behaviour. This concept is analogous to the evolution of particular organismal shapes. In such a setting, particular genes at key nodes in gene-regulatory networks are poised to accommodate changes that lead to the generation of forms[11]. Future work, following the approach pioneered by Seeholzer *et al.*, will

reveal the extent to which the evolution of behaviour is targeted to particular nodes in neural circuits. ■

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PALAEOCLIMATE

Ancient ice sheet had a growth spurt

An analysis of ancient coral from the Great Barrier Reef reveals that global sea level fell rapidly at the end of the last glacial period. The findings suggest that ice sheets are more dynamic than was previously thought. SEE LETTER P.603

PIPPA WHITEHOUSE

The episode known as the Last Glacial Maximum extended from 26,500 to 19,000 years ago, and is often thought of as a prolonged time during which glaciation was continuously at its greatest extent[1]. But on page 603, Yokoyama *et al.*[2] report an analysis of relict coral reefs that suggests that the already extensive ice sheets underwent a final burst of rapid growth about 22,000 years ago, at a rate sufficient to lower global mean sea level by an astounding 17 metres in only 500 years — about ten times faster than the current rate of sea-level rise. This seems to have been followed almost immediately by ice-sheet retreat. Understanding the trigger for this previously undocumented growth is crucial to understanding the sensitivity of ice sheets to external drivers and internal feedbacks.

Previous studies have tended to focus on the magnitude of sea-level decline during the Last Glacial Maximum (LGM), rather than the timing of it, with the general consensus being that sea level was about 125–135 m lower than it is today[3,4]. Geological records that shed light on the detailed timing of sea-level change during this period are notoriously difficult to obtain, not least because they are often located about 100 m underwater. To address this problem, Yokoyama *et al.* analysed a 'staircase' of relict coral reefs that once formed part of the Great Barrier Reef at the edge of the Australian continent — analysis of the recovered corals indicates the sea level at the time they were alive, whereas the reef age was determined using radiometric dating techniques.

One issue with such studies is that modern corals live at a range of depths, creating considerable uncertainty about the depths that can be inferred from fossilized samples[5]. However, by piecing together information from adjacent reefs at different depths, Yokoyama *et al.* built up a composite picture of the timing and magnitude of LGM sea-level decline off the coast of Australia. Their approach is clever and simple: they reason that a hiatus in coral growth caused by sea-level fall at one site must coincide with the development of a second, nearby reef farther offshore, as water depths became shallow enough to support growth at the deeper site (Fig. 1). Moreover, samples recovered from the second site indicate that the sea-level minimum (the lowstand) was short-lived, and that sea level soon began to rise again.

To identify the ice sheets responsible for the dramatic sea-level fall, Yokoyama *et al.* turned to a technique known as glacial isostatic adjustment (GIA) modelling, which can predict how ice-sheet change translates into sea-level change[6]. Although increases in ice mass directly equate to decreases in ocean mass, the resulting pattern of sea-level change is not straightforward. This is because the land beneath ice sheets subsides as mantle material is displaced by the weight of the accumulating ice, and rebounds when the ice melts; an equivalent process takes place across ocean basins in response to increases or decreases in ocean mass. The changes in the shape of the solid Earth alter the shape of the planet's gravity field. And because the gravity field defines the

50 Years Ago

Compensation of £3,200 has so far been paid by the Ministry of Technology for the damage caused by the four sonic boom tests carried out over London last summer with Lightning aircraft. The other seven tests over other parts of Britain cost just over £700 in compensation … With one exception, all the claims involved damage to property. The exception was a claim made by a woman from Hornchurch who suffered a partial loss of hearing … and accepted £150 settlement. Even before the tests, the British Government seems to have recognized that aircraft should not be allowed to fly at supersonic speeds over densely populated areas … It seems inevitable that all supersonic flight over land will be prohibited, at least until a great deal more information on the effect of sonic booms has been collected.
From *Nature* 27 July 1968

100 Years Ago

An article on coal-saving … appears in *Engineering* for July 12. The author … gives average figures for 250 typical steam-boiler plants, covering … 1910 to the present time. It is estimated that 58,500,000 tons of coal per annum are used in this country for steam-raising purposes … exclusive of 15,000,000 tons used in railways. The 250 plants had a total of 1000 boilers, principally of the Lancashire type. With hand-firing the average net working efficiency is 57.8 per cent., as against mechanical firing with an average net working efficiency of 61.4 per cent. … The author estimates that there are 45,000 to 60,000 steam boilers at work in Great Britain, calculated in terms of averaged-sized Lancashire boilers, and considers that all the steam produced in the country to-day could be obtained much more economically with 25 per cent. fewer boilers.
From *Nature* 25 July 1918