mouse that had a T-cell-precursor-derived cancer.

There are several possible reasons why the *in vivo* and *in vitro* results differ. *In vitro*, a single group of precursor cells was followed to a particular time point in development, whereas, *in vivo*, multiple groups were present that would have been in the thymus for different lengths of time. Therefore, *in vivo*, short-lived cell types would be under-represented and aberrant cells might have been removed in the thymus by scavenger cells called macrophages.

Furthermore, other microenvironmental differences might limit the generation of abnormal cells *in vivo*, if, for example, thymic stromal cells expressed a wider range of alternative β-selecting ligands, beyond MHC. Such ligands have not been identified. The differences might also occur because fetal cells were used *in vitro* and adult cells were analysed *in vivo*, and fetal and adult precursor cells have different developmental features[16–18].

Duke-Cohan *et al*. found intriguing evidence for a possible mechanism that could compensate *in vivo* for the lack of conventional MHC. The thymic immune cells in MHC-deficient mutant mice had higher-than-normal expression of 'non-classical' MHC molecules, which are related to, but different from, conventional MHC molecules. Recognition of such ligands on fellow T-lineage cells by DP cells at a later stage can direct an alternative form of positive selection[19]. Conceivably, a high level of non-classical MHC might also provide an alternative ligand for β-selection.

The authors' results indicate that interactions between MHC and pre-TCR in β-selection can shape the TCRβ repertoire of DP cells before positive selection, whereas it is usually assumed that DP cells are developmentally equivalent before this step. But in the *in vitro* system that the authors used to study differentiation, only interactions with a type of MHC called class I could occur, whereas in the normal thymus, another type of MHC (class II) might also have a role in β-selection. After β-selection, during positive selection, the MHC class recognized by TCRαβ is known to direct 'effector' fate choices for T cells (whether these cells become helper or killer T cells). Would TCRβ chains selected on MHC class I cause the whole mature TCR complex to be biased towards class I in terms of their preferred type of MHC interaction partners, even after these TCRβ chains have paired with random TCRα?

If so, then the DP population emerging from β-selection might be a mosaic of cells with different TCRβ repertoires based on the MHC that selected them, and potentially biased to alternative effector-fate preferences already. Thus, β-selection might influence the developmental identities of T cells, both through confirming the completion of the earlier commitment step and through a possible influence on the direction of the later positive-selection step.

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Behavioural neuroscience

A neural strategy for directional behaviour

Daniel Tomsic & Jamie Theobald

How does a fruit fly's brain determine which way the animal should escape in the face of a looming predator? A mechanism involving numeric gradients of synaptic connections between neurons provides an answer. **See p.534**

Eyes are essential for revealing details that would otherwise stay hidden – even quiet or odourless objects cannot usually avoid interacting with light. The brain then uses this information to identify where an object is and decide whether it is desirable or repellent, ultimately transforming the visual patterns into the muscle activations that define a behavioural response. Topographic representations of visual space in the brain have been well documented in various species[1,2], but it remains uncertain how neural circuitry converts object locations into directional behaviour. On page 534, Dombrovski and colleagues[3] describe a pattern of neural connectivity that accounts for some such transformations, allowing fruit flies (*Drosophila melanogaster*) to escape looming visual threats. This might be a common mechanism for turning sensory input into appropriate motor actions.

Studies of fruit flies have allowed researchers to uncover the mechanisms underlying many neural computations, such as elementary motion detection[4] and the regulation of circadian rhythms[5]. Now, to understand the directional implementation of escape behaviour in flies, Dombrovski *et al*. have combined genetic tools with behavioural analyses, neurophysiology and high-resolution anatomical images. In brief, they have found that topographic representations of visual space in the retina – formed by visual projection neurons (VPNs) – are transformed by gradients of the numbers of synaptic connections VPNs make with specific 'descending' neurons that can generate directional motor output.

When a resting fly sees an object approach (a predator, for instance), it takes off in a sensible direction – backwards if the object is looming in front of the fly, or forwards if it is behind (Fig. 1). The retina conveys the position of the looming object through retinotopic columnar neurons in the first two of four optic neuropils – regions of the brain's optic lobe that contain densely packed neuronal processes called axons and dendrites (which send and receive signals to and from other neurons, respectively). The signal is then passed to VPNs in the third optic neuropil, specifically, to a type of looming-sensitive VPN termed LC4. The dendrites of each LC4 cell (of which there are roughly 70 in each half of the brain) gather information from a small visual area known as the receptive field, which for each neuron has a diameter of 20° to 40° (ref. 6). Together, the receptive fields of the LC4 neurons cover the fly's field of view.

In turn, LC4 cells project their axons towards

the midbrain to form synapses with subsets of premotor descending neurons. Dombrovski *et al.* used optogenetics (a technique to control the activity of neurons with light) to activate various descending neurons, and identified two that promote opposite take-off directions. A neuron called DNp11 evokes forward take-offs, whereas DNp02 (together with DNp04) evokes mainly backward take-offs.

In principle, a neuronal circuit that contains space-specific, dedicated neural connections (or 'labelled lines') could generate appropriate directional responses to looming stimuli[7]. A labelled-lines circuit would mean that information from each region of visual space would channel only to specific premotor neurons. Frontal looming, for example, would be conveyed only to the neurons involved in backward take-off. In this scenario, LC4 cells that gather information from forward-looking receptive fields would form synapses only with DNp02, and those with backward-looking receptive fields only with DNp11.

But the investigators found that this was not the case. Instead, both the LC4 cells responsive to frontal looming and those responsive to rear looming form synapses with both DNp02 and DNp11. The profile of synaptic connections, however, was different. By counting synapses in serial sections of fruit-fly brains, the authors found that LC4 cells that have receptive fields located towards the front of the visual field form more synaptic connections with DNp02, and progressively fewer synapses with DNp11. Conversely, LC4 cells with receptive fields towards the rear form more synaptic connections with DNp11, and progressively fewer synapses with DNp02. This suggests that antiparallel gradients of synaptic numbers between looming-sensitive neurons and premotor neurons transform retinal maps of object positions into motor coordinates to achieve the correct escape direction.

This type of 'connectomics' analysis provides fundamental information about neuronal connectivity. But the number of synaptic contacts does not necessarily reflect the strength of communication between neurons, because individual synaptic 'weights' can differ greatly[8]. To investigate whether the synapse numbers actually correlate with connection strength, the authors recorded the responses of DNp02 and DNp11 *in vivo* to looming stimuli (expanding discs of shadow projected onto a dome around the fly) presented at different locations along the front-to-rear axis of the fly's visual field. In agreement with the synaptic-number gradient, DNp11 responded more strongly to rearward than to forward stimuli, and DNp02 responded in the opposite way.

Finally, to assess whether a visuomotor transformation using synaptic gradients is exclusive to this particular system, or instead represents a general circuit-wiring design in fruit flies, the authors analysed the connectivity profiles of 20 other VPN types. Across all 20, synaptic gradients reflected the stimulus position on both the horizontal and vertical axes, implying a possible general strategy for functional neural architecture.

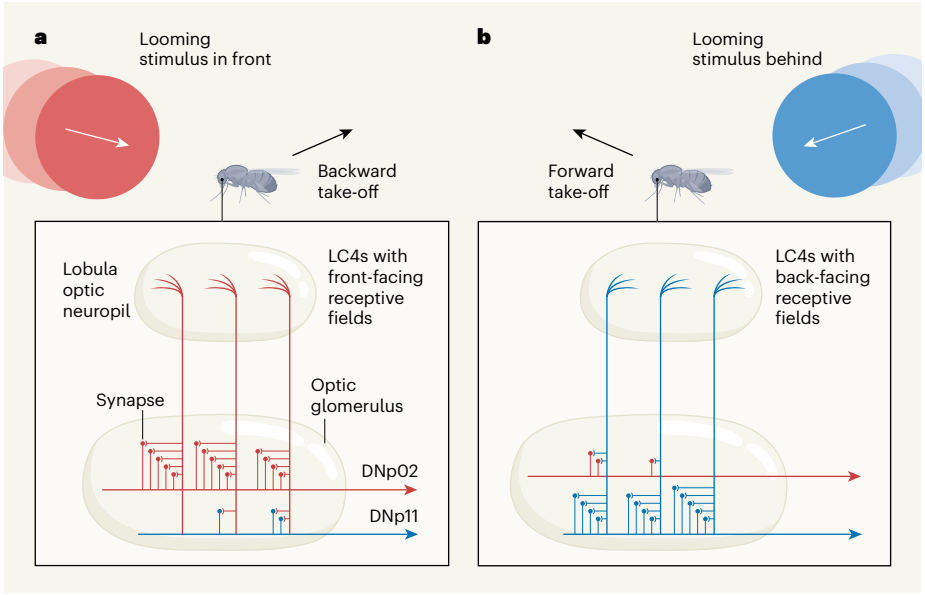

Figure 1 | Decoding object-position encoding. Approaching objects generate an expanding image in the retina known as a looming stimulus, which signals an impending threat. **a**, When a fruit fly sees a looming stimulus at the front, it takes off backwards. Retinal information about looming-stimulus position is conveyed to LC4 neurons in a brain region called the lobula optic neuropil, with each neuron receiving information about a small portion of the fly's visual field. LC4 neurons then channel the information to a structure called the optic glomerulus, where these neurons make synaptic connections with two descending neurons, DNp02 and DNp11. Activation of DNp02 drives backward take-offs, and DNp11 drives forward take-offs. Dombrovski *et al.*[3] find that gradients in the numbers of synapses between LC4 and these two descending neurons convert visual information into directional behaviour. LC4s that receive information from the front of the fly make most connections with DNp02, and least with DNp11. **b**, More-rear-facing LC4 neurons make progressively fewer connections with DNp02, but more with DNp11. Activation of these LC4 neurons in response to a looming stimulus behind the fly leads to a forward take-off.

What would be the advantage of a synaptic gradient over dedicated labelled lines? One possibility discussed by the authors is that it could enable experience-dependent adaptation. Although they think that the synaptic gradients in the system they studied are genetically determined, they speculate that, in more-flexible brain areas, this wiring design might provide a mechanism for neural plasticity. Adding and removing synapses or changing their weight would be faster and more economical than rewiring a circuit based on labelled lines. And we suggest another possible advantage: the potential for greater accuracy, because antiparallel synaptic gradients might allow the positions of visual stimuli on the retina to be transformed into directional behaviour more gradually than would be possible with labelled-line circuits.

The use of synaptic gradients for converting retinal locations into directed motor actions might represent a general neural mechanism, present in other animals and regulating other behaviours. This invites a host of possible follow-up studies to determine how widespread and varied such systems might be.

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