NEWS & VIEWS

DEVELOPMENTAL NEUROSCIENCE

Two gradients are better than one

Liqun Luo

Wiring up retinal neurons to the correct brain region during development is a feat of precision growth. A novel directional cue repels retinal neuron fibres, acting as a counterbalance to a known attractive signal.

Our brain is made up of maps that organize what we sense. In the visual system, for example, an object is represented by the spatial activation pattern of retinal ganglion cells (RGCs), which form a two-dimensional sheet in the retina. RGC nerve fibres (axons) project into the brain in an orderly manner along both the *x* and *y* axes, such that the two-dimensional image is recapitulated in the optic tectum region of the brain (Fig. 1). But how do maps like this form during development? Although the wiring diagram for how the RGC axons connect up with the tectum — the retinotopic map — has been extensively studied, it is still not completely understood[1]. On page 31 of this issue, Schmitt *et al.*[2] identify one of the signals that direct nerve fibres from the retina to their destination in the brain.

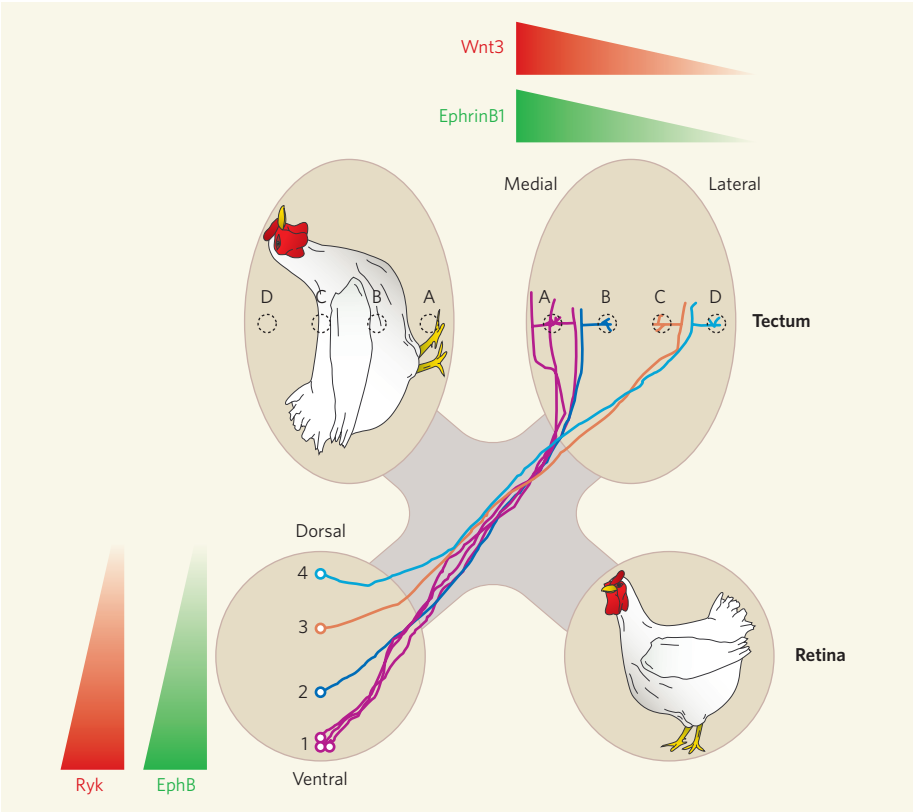

Figure 1 | Setting up the retinotopic map. An object on the retina (a chicken) is recapitulated in the tectum region of the brain. (The right retina is wired up to the left side of the brain and vice versa.) This is made possible by growth of nerve fibres (axons, coloured lines) from retinal ganglion cells (RGCs, coloured circles in the retina) along both *x* and *y* axes to the termination zones in the tectum (dotted circles). Only the retinal *y* axis, from dorsal to ventral, is depicted here. For instance, the purple neurons at the bottom perceive the bottom of the image, the chicken's feet; in the brain, they are wired up to region A on the medial side of the tectum, so the information from the image is transformed from the dorsal–ventral axis of the retina to a medial–lateral axis in the brain. The axons home in on their termination zones by regulating the direction of their branching — allowing the three purple axons to reach the same termination zone A. To achieve this, RGC axons expressing EphB receptors are attracted by the EphrinB gradient[6]. So, the purple axons with the most receptors are attracted to the medial side, where there is the most EphrinB. Schmitt *et al.*[2] have added a second gradient to this map, made of Wnt3 and its receptor Ryk. Repulsion of branches mediated by a Wnt3–Ryk gradient counterbalance the attraction mediated by EphrinB–EphB. (Adapted from refs 2 and 14.)

The retinotopic map was first elaborated by Roger Sperry 42 years ago[3]. By following point-to-point connections made as frog RGC axons regenerated between the retina and tectum, Sperry postulated that individual RGC axons must carry chemical tags that allow them to read the positional information in the tectum, also of a chemical nature. To limit the number of different tags needed to specify the connections, Sperry further proposed that the chemicals on RGC axons and in the tectum form gradients, such that the amounts of a tag could specify different positions. These ideas have been borne out spectacularly by experiment: first in the anterior–posterior axis, where gradients of a family of molecules called EphrinA in the tectum specify where RGC axons will end up[4,5]; and more recently in the medial–lateral axis, where gradients of EphrinB molecules organize how the RGCs are wired up[6,7].

In the chick and the mouse, RGC axons home in on their exact targets along the medial–lateral axis in the tectum primarily by regulating the direction of branches that extend from the primary axons[1]. When the primary axon ends up in a position lateral to where it should be, it projects branches medially to link up with its 'termination zone'; conversely, if the primary axon is medial to the termination zone, it branches out laterally (for example, the three purple neurons in Fig. 1). Graded expression of EphrinB molecules in the tectum and their receptors, the EphBs, in RGCs (Fig. 1; green gradients) regulate this direction of branching. RGCs that originate from ventral-most retina (Fig. 1; point 1) end up at the highest concentration of EphrinB (point A) because these RGCs have the most receptors, and therefore receive the most of the attractive EphrinB signal. In mutant mice that lack EphB2 and EphB3, individual axon branches preferentially extend laterally regardless of the position of primary axons relative to their termination zone, causing a lateral shift in RGC axon targeting[6].

The directions provided by EphrinB/EphB alone, however, are not sufficient to account for the medial–lateral map. If they were, all RGC axons would head for the medial-most tectum, which is most attractive, and leave the lateral tectum unoccupied. Modelling studies suggest that an additional activity, most likely a repellent gradient in the same direction as the EphrinB attractive gradient, is necessary to counterbalance the medial-directing activity of EphrinB[6]. Schmitt *et al.*[2] now show that a gradient of the Wnt3 molecule is a strong candidate for this other directional signal.

Wnt3 belongs to the Wnt family of secreted proteins — classical regulators of development that specify a variety of cell fates in embryos using concentration gradients. Wnt proteins are implicated in many biological processes[8], including axon guidance in fly embryos and in vertebrate spinal cord[9–11]. Schmitt *et al.*[2] find that Wnt3 is expressed in the tectum in a gradient of the same direction as that of EphrinB. RGC axons express two different receptors for Wnt3, called Frizzled and Ryk. Frizzled receptors seem to promote axon-branch outgrowth, whereas Ryk inhibits it. Furthermore, Ryk is expressed in a gradient in RGCs in the same direction as the EphB receptors (Fig. 1; red gradients).

Schmitt *et al.* provide two lines of evidence to support the idea that Ryk-mediated repulsion in response to the Wnt3 gradient is partially responsible for RGC axon targeting along the medial–lateral axis. First, when Wnt3 is overexpressed in the tectum, RGC axons avoid the Wnt3 expression zone. Second, when Ryk activity is compromised in RGC axons, their termination zone in the tectum shifts medially. Notably, axon branches from Ryk-compromised RGCs selectively grow medially, regardless of their location in the tectum, a phenotype opposite to RGC axons that have mutant EphB. These data strongly suggest that Wnt3 repels RGC axons through the Ryk receptor. So, the Wnt3 concentration gradient provides a laterally directing force to counterbalance the medially directing force from EphrinB on individual RGC axon branches.

This study not only adds a missing piece to the retinotopic map, but also provides a satisfactory answer to the general question of how molecular gradients can direct the establishment of a neural circuit. As mentioned above, a single gradient–countergradient of ligand–receptor is not necessarily sufficient to specify an axis, and models propose that additional forces, such as a second counterbalancing gradient or axon–axon competition, are necessary. The identification of the Wnt3 gradient provides an example of how an attractive and a repulsive gradient in the same direction work together to specify an axis of a map.

One curious finding in the work by Schmitt *et al.*[2] is the direction of the Ryk receptor gradient in RGC axons. Previous modelling suggested that this second receptor could be distributed in a gradient in the same direction as EphB, in the opposite direction, or without a gradient[6]. Given the observed direction of the gradient, and if EphrinBs and Wnt3 are the only two directional forces, then removing either EphrinBs or EphBs should cause an inversion of the map (inversion of 1→4 and A→D order in Fig. 1) in addition to a lateral shift. Mice that lack EphrinB or EphB are available, so it should already be feasible to test whether this is the case. Another prediction would be that the relative signalling strengths for the EphB and Ryk receptors differ for RGC axons originating from different positions along the dorsal–ventral axis in order to account for their differential behaviour in the tectum (imagine RGCs from points 1 and 2 in Fig. 1 arriving at the mid-point between A and B; to connect up properly, they have to branch out in opposite directions, even though their chemical environment is the same). More probably, additional directional forces may participate in these decisions, such as a potential attractive response to Wnt3 from the Frizzled receptors[2], a repulsive response to a high concentration of EphrinB[12], or activity-dependent refinement[13]. Future experiments and modelling to assess the relative contribution of these forces and how they work together will surely enrich our understanding of map formation in the brain. ■

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PLANETARY SCIENCE

The ferryman casts his shadow

David J. Tholen

The most accurate way of determining the size of some bodies in the Solar System is to observe them as they pass across the face of a star. In the case of Charon, Pluto's largest satellite, it's been a long wait.

Ask people, especially children, to name their favourite planet, and often enough Pluto crops up. Whether that is due to the association with Disney's cartoon dog, or because of the enigmatic nature of the object — uniquely among the nine traditional planets, it has never been seen at close range by a spacecraft — isn't clear. What is certain, however, is that Pluto is an oddball. It doesn't quite fit the pattern of small, rocky planets such as Earth, or large, gassy ones such as Jupiter; indeed, over the past decade, attention has seemingly been riveted on the largely semantic question of whether Pluto is a planet at all.

This debate is unfortunate, as it has overshadowed some truly significant advances. On 31 October 2005, for example, it was announced that images taken by the Hubble Space Telescope had revealed two small satellites of Pluto, to add to its already familiar moon Charon. In this issue, Gulbis *et al.* (page 48)[1] and Sicardy *et al.* (page 52)[2] detail further progress in our understanding of the cold, distant Pluto system. They present observations of a stellar occultation — the passage of a Solar System object in front of a sufficiently bright star — by Charon, allowing the most accurate assessment so far of the moon's size, and of the possibility that it has an atmosphere.

An occultation is a fairly rare, but extremely powerful, observational tool used by astronomers to make measurements of the occulting body that would otherwise be difficult or impossible to obtain. The gradual disappearance and reappearance of a star occulted by Pluto in 1988, for instance, showed that a thin atmosphere must envelop Pluto. It was 14 years before Pluto occulted a star again. When it did, it was found that its atmosphere had in the meantime approximately doubled in bulk — despite having moved farther away from the warming rays of the Sun. (Pluto has the least circular orbit of the traditional Solar System planets, its distance from the Sun ranging between 4.4 and 7.4 billion kilometres during an orbit lasting some 248 years.)

Pluto had been predicted to occult a star in 1980, but European observations confirmed that its shadow had missed Earth to the north. The recording of an occultation event at a South African observatory at the expected time therefore came as a surprise. The culprit