Neurobiology

Sculpted by competition

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Neuronal competition helps connections to form in the brain: the branches of less active neurons are more likely to retract — and, it now seems, less likely to grow — than those of their more active neighbours.

Competition pervades everyday life and sculpts our society. It is also an essential factor in the sculpting of our brains. On page 1022 of this issue, Hua and colleagues¹ provide new insight into the rules that underlie this competition.

Our brains contain billions of nerve cells that are wired together in a specific manner. During brain development, these nerve cells extend thin processes — axons — that branch and establish contacts with other brain cells. The contacts, called synapses, are sites at which neurons communicate through the release of chemical neurotransmitters. One of the most fundamental but also most complex questions in neuroscience is how the appropriate connections are formed when the brain develops. A detailed map of the vast number of synapses in the adult brain cannot be laid out in our genes: our genome is simply too small to specify such a complicated network.

It is now well established that specific molecular cues act as signposts that guide growing axons to their target cells. But the refinement of the connections depends on neural activity. A wealth of data suggests that electrical activity in developing networks influences the branching of the terminal parts (the 'arbors') of axons, and hence the formation of functional neural circuits².

Current dogma, inspired by elegant studies of the development of peripheral motor axons, holds that activity moulds the axonal arbor by regulating the extent and rate of branch retractions. Hua *et al.*¹ add to this by providing evidence that activity can also control the extent and rate of branch formation. Furthermore, their findings point to a new set of rules for activity-based competition between neighbouring axons: less active axons lose out in the competition with their more active peers by being denied the possibility of fully developing their terminal arbors.

To look at the effects of neural activity on branch formation, Hua *et al.* used the retino-tectal pathway in zebrafish as a model system (Fig. 1). This pathway is involved in the control of eye movements, and is formed by the axons of retinal ganglion cells. By transfecting individual ganglion cells with a fluorescent marker (whereby genes expressing the marker are transferred to the cell using a virus), the authors could monitor the growth and branching of single retinotectal axons by two-photon microscopy in live zebrafish. Two-photon microscopy is well suited

Figure 1 Competition in the brain. Retinal ganglion cells convey information from the retina to the optic tectum in the zebrafish brain. **a**, Individual retinal ganglion cells can be silenced by transfection with the potassium channel Kir2.1. Hua *et al.*¹ have found that the silenced axon (S) grows a less elaborate terminal arbor than do adjacent, active axons (A). **b**, Silencing of all retinal ganglion cells by injecting the neurotoxin tetrodotoxin into the eye restores the ability of the Kir2.1-expressing cell to grow a terminal arbor. Thus, competition between neighbouring axons plays a part in the growth and branching of axon arbors.

for such analyses^{3,4} because it allows many images to be obtained without interfering unduly with the growth process.

To investigate how inactive axons fared in the competition with active neighbouring ones, Hua *et al.* needed to suppress the activity of single axons. To this end, they took advantage of previous findings⁵ showing that individual neurons can be silenced by transfecting them with a specific type of potassium channel — the human inward rectifier K⁺ channel Kir2.1. Transfected cells are silenced because overexpression of Kir2.1 causes them to hyperpolarize. Hua and colleagues' key observation was as follows: axons of cells that were made to overexpress Kir2.1, and which were presumably inactive, showed reduced growth and produced less elaborate terminal arbors compared with the axons of adjacent cells (Fig. 1a).

In itself, this finding cannot be taken as evidence of competition: growth could have been impeded as a direct consequence of inactivation. This possibility was ruled out by a crucial experiment in which all axons of the retinotectal pathway were silenced by tetrodotoxin — a neurotoxin isolated from puffer fish. It turned out that this neurotoxin restored the ability of the Kir2.1-expressing

axons to grow terminal arbors (Fig. 1b). So the growth inhibition of individually silenced Kir2.1-expressing axons must be due to negative feedback — directly or indirectly — from nearby, active axons.

What could be the mechanism that explains why Kir2.1-expressing neurons lose this growth competition with normal cells? An obvious possibility is that the effects of Kir2.1 overexpression are mediated through neuronal hyperpolarization, and a consequent block of neurotransmitter release. In support of this, Hua *et al.* found that the inhibitory effect of Kir2.1 on axon growth could be reproduced by transfecting retinal ganglion cells with a modified synaptic protein that suppresses the recycling of synaptic vesicles (a crucial stage in synaptic neurotransmission). Later-acting mechanisms are less clear. One possibility is that inactive axons fall prey to inhibition by adjacent, more active axons through the release of retrograde signals from the axons' target cells in the tectum (the dorsal part of the midbrain).

Hua and colleagues' main conclusion is that activity-based competition between axons sets in at an early stage, before the axons have developed a full terminal arbor. The axons seem forced to obey the rule that if you are less active than your neighbour, you are not allowed to grow.

These findings do not overthrow the current dogma — that competition acts by way of axon retraction or elimination. Rather, they suggest that the picture is more complicated, and that relative activity influences the development of functional networks by affecting axon growth as well as axon regression. These conclusions must, however, be tempered by the fact that the experimental design — based on silencing individual axons — does not mirror the situation in the developing brain, where neighbouring axons are likely to compete on more equal terms.

Nonetheless, through their ingenious combination of techniques, Hua *et al.*¹ have provided fresh insight into the mechanisms that sculpt the developing brain. One is left wondering to what extent this newly discovered rule for axon competition applies to other neuronal pathways and other species — and whether this rule is also relevant to the ability of the mature brain to develop and change.

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