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NEURODEVELOPMENT

Pruned to perfection

During development, some synaptic connections between neurons are removed by immune cells called microglia, and others are retained. The discovery of a 'don't eat me' signal that prevents excess pruning sheds light on this process.

SERGE RIVEST

The signals transmitted between neurons through synaptic connections are responsible for most, if not all, brain functions, from learning to decision-making. During brain development, synapses that are stimulated less often than others are eliminated through a process called pruning, whereas those that are highly stimulated are retained. This refines the brain's ability to respond to stimuli and environmental cues. Microglia, the brain's innate immune cells, have a key role in pruning — they engulf and digest synapses through a process called phagocytosis. But the mechanism that determines which synapses they avoid has been unclear. Writing in *Neuron*, Lehrman *et al.*[1] describe a 'don't eat me' signal, involving a protein called cluster of differentiation 47 (CD47), that prevents inappropriate synaptic pruning by microglia.

About a decade ago, it was shown that synapses requiring elimination send an 'eat me' signal to microglia[2] (Fig. 1a). This signal involves the proteins C1q and CR3, which are part of the complement cascade — a complex series of interactions that is best known for activating cells of the innate immune system to eliminate disease-causing organisms and damaged cells. 'Don't eat me' signals act to limit the effects of 'eat me' signals in the immune system, but it was not known whether the same process occurs during synaptic pruning in the developing brain.

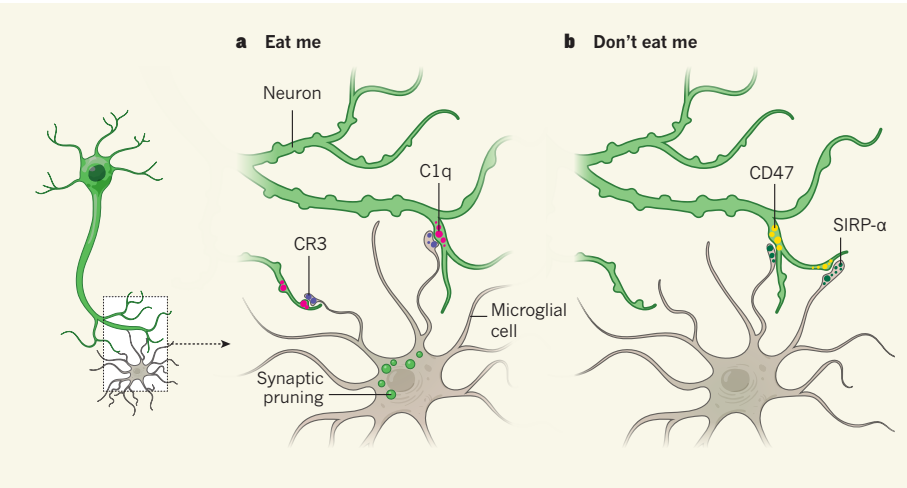

Figure 1 | Opposing signals in synaptic pruning. **a**, Unnecessary synaptic connections between neurons can be removed during brain development in a process called pruning, in which the termini of neurons leading into synapses are engulfed and digested by microglia — the brain's innate immune cells. Synapses destined for elimination release an 'eat me' signal, in which an immune protein called C1q signals to the protein CR3 on microglia to promote pruning. **b**, Lehrman *et al.*[1] report an opposing 'don't eat me' signal. The protein CD47 is expressed on active synaptic termini, and signals to its receptor SIRP-α on microglia, discouraging the immune cells from digesting the synaptic terminal.

CD47 is a cell-surface protein that has many immune functions, including acting as a 'don't eat me' signal for macrophages[3], microglia's sister cells, which exist outside the brain. Lehrman *et al.* analysed whether CD47 is expressed in the dorsal lateral geniculate nucleus (dLGN), a region of the brain involved in vision. This region receives inputs from neurons called retinal ganglion cells (RGCs) that originate in the retina. The authors demonstrated in mice that, at five days after birth, synapses from RGCs to other neurons in the dLGN are being pruned at high levels.

Lehrman and colleagues found that CD47 was expressed at higher levels in the dLGN than in other brain regions at this time. Moreover, the protein SIRP-α, which acts as a cell-surface receptor for CD47, was highly expressed by microglia at the same developmental stage. Using a super-resolution imaging technique, the researchers showed that CD47 was located in 25% of synapses in the mouse dLGN 5 days after birth.

Next, the group investigated whether CD47 functions as a 'don't eat me' signal in this context. First, they measured phagocytosis of synaptic material in mice genetically engineered to lack CD47. They found that microglia engulfed more RGC inputs in CD47-deficient mice than in their wild-type siblings. The mutant mice also displayed higher levels of pruning than did controls, and had fewer synapses in the dLGN by ten days after birth — a change that persisted into adulthood. The authors observed a similar phenomenon in mice lacking the gene that encodes SIRP-α, indicating a possible CD47–SIRP-α interaction on microglia.

The researchers used various *in vitro* approaches to test whether CD47–SIRP-α signalling could prevent the phagocytosis of isolated synaptic termini, called synaptosomes. These analyses revealed that microglia lacking SIRP-α engulfed synaptosomes more efficiently than did wild-type microglia, and that microglia preferentially engulfed synaptosomes lacking CD47 over wild-type ones. Together, these data indicate that CD47–SIRP-α signalling acts as a 'don't eat me' signal to protect against excessive microglia-mediated pruning and synapse loss (Fig. 1b).

Blocking or disrupting neuronal stimuli and environmental cues to neurons can alter synaptic pruning and refinement in

many brain regions[4]. To investigate whether activity-dependent changes in synaptic CD47 might determine which synapses are removed, Lehrman *et al.* set up an artificial competition assay, in which they used the neurotoxin tetrodotoxin (TTX) to suppress neuronal activity in one eye in mice. They found that CD47 levels were higher in synaptic inputs from RGCs that originated in the more-active control eye than in the inputs from the TTX-treated eye, suggesting that CD47 is either degraded or moves away from synapses in RGCs of the less-active eye.

Finally, Lehrman and colleagues showed that, in wild-type mice, CD47 levels were higher in more-active than in less-active synapses, and that, in CD47-deficient animals, microglia showed no preference for less-active inputs. Together, the authors' data indicate that protective 'don't eat me' signals prevent aberrant microglial engulfment in the dLGN.

As Lehrman *et al.* point out, a major challenge for the immune system is to remove dying cells, disease-causing organisms and toxic molecules without removing or damaging healthy cells. This study is particularly interesting in that regard, because the 'eat me' and 'don't eat me' signals from synapses both act on microglia. Whether the complement system and CD47–SIRP-α act separately or interact to ensure that the correct synapses are removed or protected remains an open question. This aspect is also not clear for macrophages in the immune system.

The mechanisms by which synapses that are either unwanted or to be retained send these signals to microglia remain to be demonstrated — in particular, the interaction between CD47 and SIRP-α in microglia as a leading factor in constraining synaptic pruning has not been shown directly and has been validated using *in vitro* models. The physiological relevance of this signalling pathway also needs to be examined, because CD47-deficient mice do not seem to have major defects in brain development[5].

Another question is whether these events modulate other innate immune responses in the brain, such as those involved in disease. For instance, in multiple sclerosis, a sheath of a fatty substance called myelin that insulates neurons becomes damaged. Microglia have a key role in removing damaged sheaths to enable remyelination[6]; perhaps 'eat me' and 'don't eat me' signals help the microglia to determine whether myelin should be removed.

Synaptic protection is essential for normal brain development. The identification of a 'don't eat me' signal in microglia reveals a new aspect of this crucial developmental phenomenon. ■

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ASTRONOMY

Evidence of ancient Milky Way merger

An analysis of data from the Gaia space observatory suggests that stars in the inner halo of the Milky Way originated in another galaxy. This galaxy is thought to have collided with the Milky Way about ten billion years ago. SEE LETTER P.85

KIM VENN

From studying the exquisite images collected by ground- and space-based telescopes over the past century, astronomers have learnt that galaxies can collide. On page 85, Helmi *et al.*[1] use data from the Gaia space observatory to determine that the Milky Way was hit by a satellite galaxy roughly ten billion years ago. Stars from this galaxy are still around us today to tell the story.

Gaia was launched in 2013 by the European Space Agency as the successor to Hipparcos — a satellite that in 1997 produced the first high-precision catalogue of nearby stars[2]. Gaia was designed to conduct ongoing observations of the visual characteristics and positions of more than one billion objects in the sky[3] (Fig. 1). Such map-making might seem like tedious work, but repeated measurements made by Gaia can also be used to determine precise distances and velocities across the sky for about 1% of all the stars in our Galaxy[3].

The information from Gaia can be combined with spectroscopic measurements of velocities along the observer's line of sight to make videos that show the precise motions of the stars (see, for example, go.nature.com/2atris8). Playing these videos backwards allows astronomers to study how our Galaxy was assembled and how it has evolved.

Helmi and colleagues used the Gaia mission's second data release, which was published earlier this year[4], to analyse the motion of stars near the Sun (within a distance of about 10 kiloparsecs). The authors compared these observations with predictions from simulations in which the Milky Way and a satellite galaxy with 20% of the mass of our Galaxy merged in the past[5]. The similarities are striking, particularly the detailed motions of some high-velocity stars that orbit the Galactic Centre in the opposite direction to the Sun.

Using astronomical-data catalogues that provide the ages and chemical compositions of stars[6], Helmi *et al.* determined that the Milky Way's inner halo — a region that surrounds the thick stellar disk — is made up mainly of stars from the satellite galaxy. These stars provide a record of the galactic collision, which the authors estimate took place approximately ten billion years ago.

Several other research groups working with the Gaia data have reached a similar conclusion to that of Helmi and colleagues, using other analytical methods or data catalogues[7–9]. However, there are small differences between the results of Helmi *et al.* and those of the other groups, such as the mass of the satellite galaxy, when the collision occurred and whether the event involved a single satellite galaxy or a few smaller galaxies.

One conclusion on which all of the groups agree is that the event might have contributed to the formation of the Milky Way's thick stellar disk. If a thin disk of stars encircled the Galactic Centre at the time of the merger, the orbits of the stars would have been disrupted. Originally, the stars would have had a specific chemical composition that reflected the young age of the Milky Way. But today, they would be old and relatively poor in metals (elements heavier than helium), and on orbits in a thickened disk. All the research groups reported the possible identification of these old stars in the Gaia data.

Astronomers have speculated for several decades that an ancient satellite galaxy merged with the Milky Way in the past, because such an event could explain differences in the motions and chemical compositions of stars in the neighbourhood of the Sun. For example, one of the most unusual objects in our Galaxy is Omega Centauri — a cluster of stars so distinctive that it is thought to be the core of a satellite galaxy that was disrupted and absorbed by the Milky Way. Researchers have suggested that some of the stars found in the Gaia data might be debris from this event[10,11].

Obtaining proof that some stars are associated with a merger required the high precision and large survey area of Gaia, in combination