Microglia tweak retinogeniculate pathways during visual circuit refinement

Tara Keck
Department of Neuroscience, Physiology and Pharmacology, University College London, 21 University St., London, WC1E 6DE, UK
Correspondence: t.keck@ucl.ac.uk

Abstract: Cheadle et al. reveal that microglia expressing TWEAK facilitate synapse elimination through a novel, non-phagocytic mechanism in the retinogeniculate pathway during visual circuit development. This novel mechanism is experience-dependent and occurs through the local binding of TWEAK to postsynaptic Fn14.

Sensory-dependent plasticity plays a critical role in the development of the visual system. While molecular cues and spontaneous activity are essential for establishing connections early in development, visual experience is critical for the precise refinement of these circuits in the later stages (Hooks and Chen, 2006). The retinogeniculate pathway has been used extensively as a model to examine both components of circuit development. To date, a number of mechanisms have been described for the early stages of development (Huberman, 2007), but the molecular mechanisms associated with experience-dependent refinement are less clear.

To examine the molecules that regulate synaptic refinement, previous work from Cheadle et al. used single-nucleus RNA sequencing to demonstrate that the cytokine receptor fibroblast growth factor-inducible protein 14kDa (Fn14) was induced in excitatory thalamocortical neurons in response to visual stimulation during experience-dependent visual development (age p20-p27) (Cheadle et al., 2018). Using electrophysiology and ultrastructural methods, they showed that the strengthening of connections between retinal ganglion cells (RGC) and thalamocortical neurons required Fn14. Synapses were not strengthened as expected either in Fn14 knockout (KO) mice or following sensory deprivation, which resulted in a reduction of Fn14 induction (Cheadle et al., 2018). Furthermore, the absence of Fn14 through genetic manipulation or deprivation also reduced the

elimination of weak synapses that occurs during circuit refinement (Lin et al., 2014). The effects of manipulating Fn14 were only present during the later period of visual development (between p20 and p27), but not prior to p20. While this study highlighted the important role of Fn14 in experience-dependent visual refinement, how Fn14 induction differentially regulated synapse strengthening and elimination remained unclear.

In this issue, the authors build on their previous work to describe the mechanism underpinning the opposing Fn14 effects on the retinogeniculate connections (Cheadle, 2020). They used a novel form of semi-automated serial transmission electron microscopy (TEM) to examine the structural properties of synapses during this period of experience-dependent visual development. Consistent with their previous work (Cheadle et al., 2018), the authors found that synaptic strengthening and elimination is dependent on Fn14. In the absence of Fn14, the total percentage of bulbous spines (mushroom-like spines with a spine head that is at least twice as wide as the neck) receiving RGC inputs decreased from 60% to 30%. Furthermore, in wildtype mice, bulbous spines on average have four synapses per spine, compared to one to two synapses per spine in thin and non-bulbous spines. In mice where Fn14 was eliminated via genetic manipulation or sensory deprivation, the bulbous spines had 40% fewer synapses per spine. This Fn14-dependent effect was largely confined to bulbous spines, with little effect on the number of synapses in thin or non-bulbous spines. Together, this decrease in both the number of bulbous spines and the number of synapses on each bulbous spine in the absence of Fn14 corresponds to a strong decrease in the total RGC input to these neurons when Fn14 function is disrupted. Additionally, the authors developed an Fn14 conditional KO mouse, which allowed them to spatially localize the effect of Fn14. They found that postsynaptic expression of Fn14 combined with sensory experience was necessary for the development of bulbous spines, with limited contribution from presynaptic effects.

Having characterized the effects of Fn14 on dendritic spines, the authors examined the molecular mechanisms facilitating this effect. The cytokine tumor necrosis factor (TNF)-like weak inducer of apoptosis (TWEAK), is a member of the TNF superfamily and binds to Fn14 (Wiley and Winkles, 2003). The authors first showed that TWEAK

is expressed in a subset of microglia in the thalamus in an experience-dependent manner. Seventy percent of microglia express TWEAK after visual stimulation, whereas only 8% express TWEAK in unstimulated mice. Furthermore, TWEAK was shown to bind in a highly localized way to retinogeniculate synapses expressing Fn14. In the absence of Fn14, TWEAK was no longer reliably detectable at synapses.

The authors then examined the effect of the TWEAK-Fn14 interaction on spines. They found that overexpression of soluble TWEAK via an adeno-associated virus (AAV) injection resulted in a 25% decrease in the bulbous spine population. Conversely, in TWEAK KO mice, the bulbous spine population was increased by 30%. The increase in spine percentage in the KO mice could be reduced to wildtype levels through re-expression of TWEAK via viral injection. Together, these results suggest that TWEAK acts locally through Fn14 to facilitate the elimination of bulbous spines.

The authors next examined how TWEAK-expressing microglia may facilitate synaptic elimination. Previous work has described mechanisms by which microglia are involved in synaptic pruning, with phagocytic engulfment as one of the best-studied mechanisms of microglial-synaptic interactions (Schafer et al., 2013). The authors examined if the TWEAK-mediated elimination of synapses occurred through phagocytic engulfment. They found that while presynaptic components of the synapse did undergo phagocytic engulfment, postsynaptic spines did not. These results suggest that TWEAK acts through a non-phagocytic mechanism. Additionally, there was no difference in phagocytic engulfment in wildtype or TWEAK KO mice, either during early (P7) or experience-dependent development (p27), suggesting that TWEAK is not involved in phagocytic engulfment. Together these results point to microglia acting through a novel experience-dependent mechanism.

Postsynaptic Fn14 is necessary for synapse strengthening and, through interactions with TWEAK, elimination. The authors next examined how the TWEAK-Fn14 interaction targets particular synapses for elimination. They found a spatial organization of spines that are strengthened or eliminated. Spines that were in the vicinity of microglia expressing TWEAK were eliminated. Specifically, within 200 nm

of a given microglial cell, there was an inverse correlation between TWEAK expression levels and the number of bulbous spines. These results suggest that there is a spatial element to synapse elimination. Whether additional genetic or experience-dependent influences determine the position of TWEAK expressing microglia with respect to the dendritic spines will require further study.

In summary, Cheadle et al. (2020) show that microglia play a novel role in shaping synaptic organization during the experience-dependent phase of retinogeniculate circuit refinement. In the early phase of visual development (prior to p20), synaptic changes occur that are independent of Fn14-TWEAK signalling. After p20, there is an experience-dependent expression of Fn14 in the thalamic relay neurons and TWEAK in microglia. In this later phase, microglia employ an experience-dependent process to sculpt postsynaptic spine morphology and pruning ((Cheadle, 2020) Figure 8).

While the results presented here occur during late visual development, many immune-related molecules have ongoing roles in plasticity throughout the brain (Fourgeaud and Boulanger, 2010). Thus, the plasticity mechanism described here may generalize to other developmental periods and brain regions. In line with this, TNF-alpha, which is also from the TNF superfamily and released locally from microglia, is known to be critical for homeostatic synaptic strengthening throughout development and adulthood (Barnes et al., 2017; Pribiag and Stellwagen, 2014). The degree to which the experience-dependent plasticity mechanisms described here reflect homeostatic or Hebbian processes remains to be seen, but the work adds to the literature suggesting that this class of immune-related molecules may have an important role in plasticity. Overall, the work from Cheadle et al. suggests a novel role for microglia in shaping the synaptic connectivity and organization of neural circuits, providing further evidence for the importance of this cell type in the development and plasticity of neural circuitry.

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