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SPOTLIGHT

Neuronal cadherins: The keys that unlock layer-specific astrocyte identity?

Haley Jetter[1] and Sarah D. Ackerman[1]

An astrocyte's intricate morphology is essential for proper brain function, but the intrinsic and extrinsic cues that set astrocyte morphology are largely unknown. In this issue, Tan et al. (https://doi.org/10.1083/jcb.202303138) show that layer-specific expression of neuronal cadherins locally regulates astrocyte morphogenesis and heterogeneity.

The brain is made up of billions of cells, including neurons and glia. Neurons form specialized connections, or synapses, to communicate with each other through electrical signals. These connections are critical for the formation of neural circuits that execute many functions to allow us to sense and respond to our environment. Neurons, however, cannot function without the support of glial cells. In the central nervous system (CNS), these include astrocytes and oligodendrocytes (the macroglia), as well as microglia. While glia do not send electrical signals, they are key regulators of neuronal signaling, and loss of all glia is incompatible with life. Astrocytes, which are the focus of this study, are the most abundant glial cell type in the CNS and are defined by their intricate morphology. Mature astrocytes have highly branched processes that interact with hundreds to thousands of synapses simultaneously. This close apposition to synapses is critical for their function. At the synapse, they can accelerate and "brake" the formation of new synaptic connections, they regulate synapse maturation and function, and recently they have been shown to alter patterns of neuronal signaling (e.g., gliotransmission) (1, 2). Thus, the morphology of astrocytes is essential for their function, yet we know almost nothing about the basic mechanisms that regulate astrocyte maturation and synapse association. The study by Tan et al. provides valuable insights into how neuronal cues may contribute to astrocyte morphogenesis (3).

Previous work from the Eroglu lab identified neurexins and neuroligins (NLGNs) as key for astrocyte morphogenesis (4). They showed that during mouse brain development, astrocyte morphological maturation peaks with cortical synaptogenesis. These data demonstrated that astrocyte and neuronal maturation are temporally linked and may therefore reciprocally regulate one another. The authors found that astrocyte-specific knockdown of *Nlgn1*, 2, or *3* resulted in a decrease of astrocyte branch complexity, volume of fine processes, and territory size, while overexpression of *Nlgn1* or *Nlgn2* increased astrocyte territory size. Through conditional, sparse knockout (cKO) of astrocytic *Nlgn2* in vivo, the authors demonstrated that local loss of *Nlgn2* reduced excitatory synapse number by half. Functionally, this cKO caused a reduction in circuit excitation (decreased frequency and amplitude of miniature excitatory postsynaptic currents) and enhanced circuit inhibition (increased frequency of miniature inhibitory postsynaptic currents), which changed the overall balance of excitation to inhibition (4). Thus, changes in astrocyte morphology can dramatically alter neuronal circuit structure and function.

In this previous study, the authors found that NLGN-dependent increases in astrocyte morphology rely on direct binding with neuronal neurexins. Interestingly, astrocytes exhibit layer-specific morphologies in the mouse cortex, displaying differences in both territory size and complexity (5, 6). For example, layer I astrocytes have the smallest territory size, while layer II/III astrocytes have the largest (5). Can neurons provide regionalized cues to regulate differences in astrocyte morphogenesis across cortical layers? In Tan et al., the authors answer this question by exploring the role of δ-catenin, a member of the p120-catenin subfamily of proteins, in astrocyte morphological maturation. Previous studies focused on the role of δ-catenin in neurons and identified this protein as a key regulator of dendrite morphology (7, 8). Here, the authors challenged previous assumptions that δ-catenin is expressed solely in neurons. Using RNA sequencing, western blot, immunohistochemistry, and fluorescent in situ hybridization, the authors showed that δ-catenin protein and mRNA are localized to astrocytes in the mouse primary visual cortex, and that δ-catenin levels peak with synaptogenesis. To demonstrate a causal link between δ-catenin and astrocyte morphogenesis, the authors used an in vitro neuron–astrocyte co-culturing system. The authors previously showed that co-culture of astrocytes with neurons is required for astrocytes to achieve a mature morphology in vitro, and that this system allows for

[1]Department of Pathology and Immunology, Brain Immunology and Glia Center, Washington University School of Medicine, Saint Louis, MO, USA.

Correspondence to Sarah D. Ackerman: sarah.ackerman@wustl.edu.

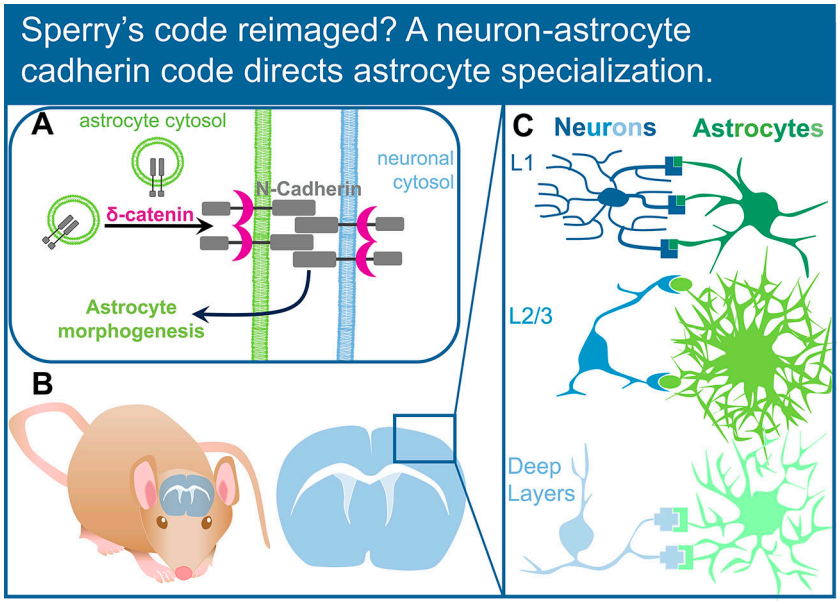

Figure 1. **Sperry's code reimaged? A neuron–astrocyte cadherin code directs astrocyte specialization. (A)** δ-Catenin facilitates surface localization and binding of N-cadherin between astrocytes and neighboring deep-layer neurons, and in turn, drives astrocyte morphogenesis. **(B and C)** In mouse, cortical layers display regionalized differences in neuronal identity and astrocyte morphology. Neuronal N-cadherin serves as a local cue to drive the distinct morphology of deep-layer astrocytes, suggesting that neuronal cadherins may represent the keys that unlock layer-specific astrocyte morphologies.

identification of genes required for neuron contact-dependent astrocyte maturation (4). To manipulate δ-catenin, they transfected cultured rat astrocytes with control short hairpin RNA (*shControl*) or short hairpin RNA to silence δ-catenin (*shCtnnd2*). When plated on wild-type neurons, *shCtnnd2* astrocytes showed significantly reduced arbor complexity compared to *shControl* astrocytes, and this phenotype could be suppressed by astrocyte-specific overexpression of full-length human δ-catenin in *shCtnnd2* astrocytes. Transfection of astrocytes in layer 1 (visual cortex) with *shCtnnd2* also reduced astrocyte complexity compared to *shControl* in vivo. Taken together, these results demonstrate that astrocytic δ-catenin is critical for neuron contact-mediated astrocyte morphogenesis in vitro and in vivo (Fig. 1 A).

Given that δ-catenin is an intracellular protein, the authors next questioned how δ-catenin might regulate astrocyte–neuron contacts. δ-Catenin is known to bind cadherins, which mediate cell–cell interactions in many biological contexts (9–11). Using structural modeling, they predicted that the juxtamembrane domain (JMD) of N-cadherin binds a positively charged groove in δ-catenin's armadillo (ARM) repeat domain. The authors confirmed this relationship through co-immunoprecipitation assays and showed that removal of the N-cadherin JMD eliminated binding with δ-catenin. The authors also identified disease-associated mutations in δ-catenin, including R713C, an autism-linked point mutation predicted to neutralize the charge of the binding groove within the ARM domain. Co-immunoprecipitation revealed that R713C does not impact the ability of δ-catenin to bind with N-cadherin; instead, R713C δ-catenin disrupted trafficking of N-cadherin to the cell surface, where it is required to mediate astrocyte–neuron contact. Accordingly, overexpression of human R713C failed to rescue morphological complexity in *shCtnnd2* astrocytes. Together, these data indicate that direct binding of δ-catenin to N-cadherin is essential for N-cadherin cell surface localization, and in turn, astrocyte morphogenesis.

Cadherins are a diverse family of proteins with regionalized expression patterns thought to be critical for local assembly of neural circuits (10, 11). Might local differences in cadherin levels and/or identity govern layer-specific astrocyte morphologies? To test this hypothesis, the authors silenced δ-catenin in upper-layer, but not lower-layer neurons, which should reduce surface levels of cadherins specifically in upper-layer neurons. As expected, this resulted in reduced complexity and volume of upper-layer astrocytes with no change in the morphology of lower-layer astrocytes. Thus, regionalized changes in neuronal cadherins can alter astrocyte morphology. Furthermore, while N-cadherin is expressed in all cortical astrocytes, it is restricted to lower-layer cortical neurons, suggesting that N-cadherin may instruct the unique morphology of lower-layer astrocytes. Indeed, loss of astrocytic N-cadherin throughout the cortex only impacted the morphology of lower-layer astrocytes. These data support the hypothesis that neuronal cadherin expression is sufficient to dictate regionalized astrocyte morphology.

For many decades, neuroscientists have tried to unravel the molecular codes that instruct neural circuit assembly. In the mid-twentieth century, Roger Sperry coined the "chemoaffinity hypothesis," which suggested that synaptic partners express complementary cell surface cues that allow circuits to be assembled and reassembled in a stereotyped fashion (12). Here, Tan et al. provide significant, novel evidence that layer-specific neuronal cues regulate astrocyte morphogenesis and heterogeneity, expanding the importance of Sperry's original hypothesis to include glia (Fig. 1, B and C). In the future, it will be important to elucidate the proposed neuron-glia cadherin code, including but not limited to whether regionalized cadherin expression patterns can also regulate astrocyte functional heterogeneity between cortical layers. As astrocyte function is frequently altered in neurological diseases, understanding the mechanisms that specialize astrocyte identity is of profound clinical importance.

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