Journal Club

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Target-Derived Cues Instruct Synaptic Differentiation

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Review of Yamagata and Sanes (<http://www.jneurosci.org/cgi/content/full/25/37/8457>)

Introduction

Neural circuits comprise highly intricate and precise patterns of synaptic connectivity. How does synaptic specificity arise? A salient anatomical correlate of synaptic specificity is laminar specificity: throughout the brain and spinal cord, neurons confine their axonal and dendritic processes to particular layers, thereby limiting the number and type of synaptic partners available to them. Although much is known about how axons navigate to find their targets and form topographic maps, much less is known about the cues that induce layer-specific connectivity during development (Benson et al., 2001). A related question is whether the cues that direct layer-specific axonal targeting also instruct synaptic differentiation.

A recent article by Yamagata and Sanes in *The Journal of Neuroscience* [Yamagata and Sanes, (2005) (<http://www.jneurosci.org/cgi/content/full/25/37/8457>)] provides new insights into these questions. The authors investigated how axonal projections of retinal ganglion cells (RGCs) connect to the appropriate synaptic layers in the chick optic tectum, avian homolog of the mammalian superior colliculus. In the chick, RGC axons terminate within 3 of the 15 tectal layers. Moreover, each individual RGC axon targets just one particular retinorecipient layer, depending on RGC subtype (Fig. 1). What sorts of cues

guide RGC axons to their correct layer? The recent work of Yamagata and Sanes (2005) was prompted by their previous findings that the plant lectin *Vicia villosa* agglutinin B4 (VVA-B4) selectively binds the retinorecipient layers. VVA-B4 recognizes *N*-acetylgalactosaminyl (GalNAc) residues, cell-surface carbohydrates that are typically found as components of the extracellular matrix. In functional assays, the authors also previously established that VVA-B4 application prevents RGC axons from completely entering the retinorecipient layers but does not affect axon arborization (Inoue and Sanes, 1997). Those results suggested that specific components of the extracellular matrix are involved in layer-specific targeting of RGC axons. However, until recently, the identity of those proteins remained unknown.

To identify the tectal proteins that bind VVA-B4, Yamagata and Sanes (2005) used a biochemical approach in which they homogenized hundreds of chick tecta and applied that material to a VVA-B4-containing column. Then they isolated and purified the proteins that bound VVA-B4, by elution with GalNAc. Analysis of the purified proteins with mass spectrometry revealed that all were lectican-type proteoglycans derived from agrin, aggrecan, or versican [Yamagata and Sanes (2005), their Fig. 1 (<http://www.jneurosci.org/cgi/content/full/25/37/8457>/FIG 1)]. Which of these might regulate layer-specific targeting of RGC axons? *In situ* hybridization revealed that only versican was selectively expressed within the retinorecipient tectal layers [Yamagata and Sanes (2005), their Fig. 2 ([\[www.jneurosci.org/cgi/content/full/25/37/8457\]\(http://www.jneurosci.org/cgi/content/full/25/37/8457\)/FIG 2\)\] and RNA interference \(RNAi\)-mediated knockdown of versican eliminated VVA-B4 binding to the tectum \[Yamagata and Sanes \(2005\), their Fig. 3 \(<http://www.jneurosci.org/cgi/content/full/25/37/8457>/FIG 3\)\], giving strong indication that versican is the major tectal receptor for VVA-B4.](http://</p>
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What type of cells express versican? Although the majority of previous findings show that versican is made by glia, double-label *in situ* hybridization showed that, in the embryonic chick tectum, versican is expressed by local interneurons that also express reelin, substance-P receptor, or neuropilin-1 but not by cells expressing the glial marker glutamine synthetase. These experiments also revealed that versican expression is graded across the depth of the tectal layers [Yamagata and Sanes, (2005), their Fig. 4 (<http://www.jneurosci.org/cgi/content/full/25/37/8457>/FIG 4)] (Fig. 1), which could have implications for layer-specific axon guidance.

Does versican guide RGC axons to specific tectal layers? Apparently not. Unlike the effects of VVA-B4 seen previously, RNAi-mediated knockdown of versican did not prevent RGC axons from entering the tectum or targeting the retinorecipient layers. In fact, each different RGC subtype still targeted its correct layer [Yamagata and Sanes (2005), their Fig. 4 (<http://www.jneurosci.org/cgi/content/full/25/37/8457>/FIG 4)]. To better understand the effects of versican on RGC axons, the investigators next used retinal explant cultures in which RGC axons grew out to

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encounter stripes of versican. The results were clear: axons failed to extend past the borders of highly concentrated versican stripes. Previous experiments have already shown that versican can inhibit axon outgrowth (Schmalfeldt et al., 2000). Interestingly, Yamagata and Sanes (2005) also found that large varicosities containing the presynaptic vesicle-associated protein SV2 formed in axons selectively at the sites of contact with versican [Yamagata and Sanes, (2005), their Fig. 5 (<http://www.jneurosci.org/cgi/content/full/25/37/8457/FIG 5>)]. This shows that versican is sufficient to induce presynaptic maturation *in vitro*. Next they performed RNAi-mediated knockdown of versican *in vivo* and found that the number of large presynaptic puncta within the retinorecipient tectal layers were dramatically reduced. The effect was specific to large presynaptic profiles because the overall number of presynaptic and postsynaptic puncta was not altered [Yamagata and Sanes, (2005), their Fig. 6 (<http://www.jneurosci.org/cgi/content/full/25/37/8457/FIG 6>)]. They also directly examined the morphology of dye-labeled RGC axons and found a significant reduction in large presynaptic varicosities on RGC axons in the absence of versican [Yamagata and Sanes, (2005), their Fig. 7 (<http://www.jneurosci.org/cgi/content/full/25/37/8457/FIG 7>)]. Because large presynaptic varicosities are characteristic of mature, well differentiated RGC synapses, these findings provide additional evidence that versican mediates synaptic maturation.

Collectively, the results of Yamagata and Sanes (2005) show that versican is an important layer-specific cue for presynaptic maturation of RGCs. However, some key questions about its role in layer-specific targeting of RGC axons remain. Despite evidence that versican is the major VVA-B4 receptor in the chick tectum, why is it that VVA-B4-induced blockade of those receptors prevents RGC axons from innervating the tectum (Inoue and Sanes, 1997), whereas knocking down versican has no apparent effect on layer-specific RGC targeting? In their discussion, the authors provide several interest-

Figure 1. Schematic of RGC projections to the retinorecipient layers (shown in blue) of the chick optic tectum. Each RGC axon targets a different retinorecipient layer according to RGC subtype (different subtypes schematized as red, yellow, or green). The dendrites of neurons within the deeper tectal layers (in black) ramify in the overlying retinorecipient layers and thereby receive RGC synaptic input. Interneurons within each layer (in black) also receive RGC input. VVA-B4 binding is enriched within the retinorecipient layers. Versican is the tectal VVA-B4 receptor; it is expressed in a gradient across the depth of the retinorecipient layers (purple) by interneurons that coexpress reelin, substance P receptor (Substance PR), or neuropilin-1. For details, see the study by Yamagata and Sanes (2005). SGC, Stratum griseum centrale; SAC, stratum album centrale; SGP, stratum griseum periventriculare; SFP, stratum fibrosum periventriculare.

ing and testable explanations for this discrepancy. For instance, they propose that VVA-B4 might also have effects on RGC axons and not just tectal cells. An additional consideration, however, is that there are many different classes of RGCs, and these synapse onto highly specific subsets of postsynaptic tectal cells, including interneurons (Tombol et al., 2003). Thus, although RGC axons still targeted the correct retinorecipient layers in versican-depleted tecta, it remains possible that, within a given retinorecipient layer, RGC axons targeted the wrong postsynaptic cells. The observation that versican expression is graded across the depth of the retinorecipient layers and is expressed by molecularly distinct sets of interneurons [Yamagata and Sanes, (2005), their Fig. 4 (<http://www.jneurosci.org/cgi/content/full/25/37/8457/FIG 4>)] reinforces this idea. Thus, experiments that combine versican depletion with labeling of both RGC axons and postsynaptic tectal cells would be very informative.

The results of Yamagata and Sanes (2005) are especially noteworthy because, although molecules such as N-cadherin have been shown previously to guide axons to particular layers within their targets (Inoue and Sanes, 1997; Benson et al., 2001), there have been very few reported

instances of cues that promote synaptic differentiation in a layer-specific manner. It is tempting to speculate that the effects of versican on RGC synapses might reflect just one instance of a more general phenomenon in which the cues that promote synaptic maturation are expressed in a layer-specific manner so as to stabilize specific groups of synapses.

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