Molecular mechanisms that establish the eye-specific visual projection

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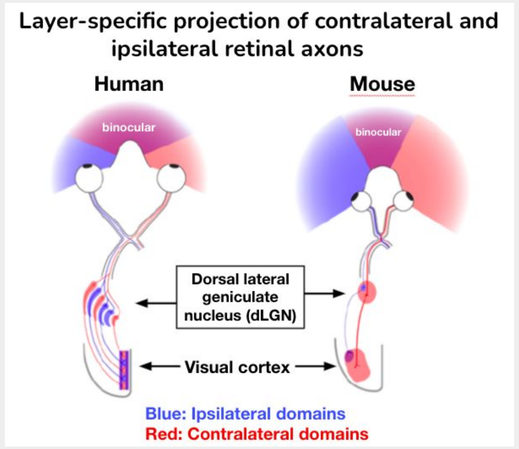
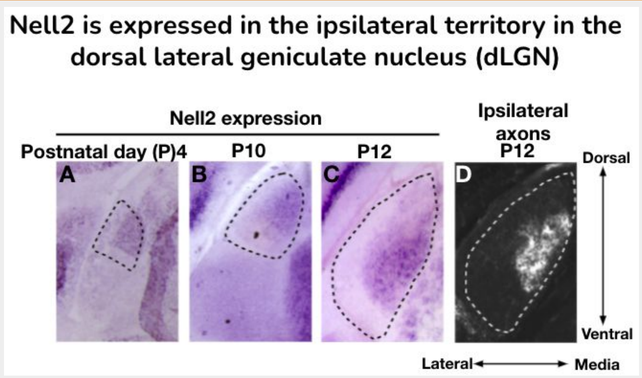
ABSTRACT

Correct functioning of the nervous system critically depends on the formation of the precise neuronal network. Axons of retinal ganglion cells from the right and left eyes project to different domains in the lateral geniculate nucleus of the thalamus. This eye-specific retinogeniculate projection provides the anatomical basis for binocular vision. Nell2 (neural epidermal growth factor (EGF)-like-like 2) is an extracellular glycoprotein that is predominantly expressed in the nervous system. Our lab has previously shown that Nell2 acts as an inhibitory axon guidance molecule in the establishment of the eye-specific retinogeniculate projection. The current work aims to identify the Nell2 receptor in retinal axon guidance. By using immunohistochemistry, we found that the receptor tyrosine kinase Ros1, which binds to Nell2, is expressed in the developing chick retinal ganglion cells. Our results suggest that Ros1 may act as a receptor for Nell2 in retinal ganglion cells and play a significant role in the establishment of the eye-specific retinogeniculate projection.

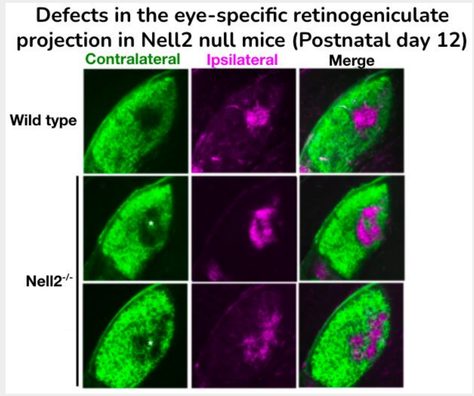
BACKGROUND



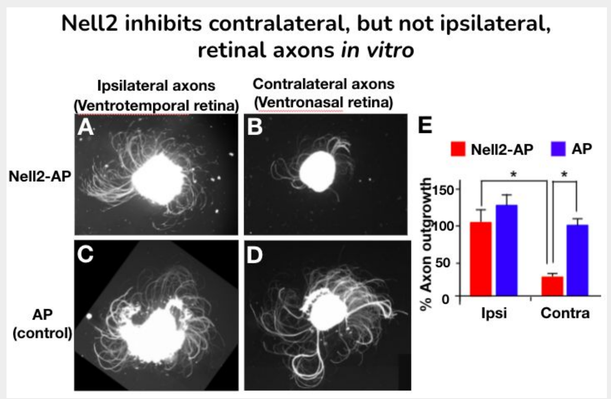
In vertebrates that have binocular vision, retinal axons project to the both side of the brain. Retinal axons from the ipsilateral (same side) and the contralateral (opposite side) eyes project to different layers/domains of the brain target, the dorsal lateral geniculate nucleus (dLGN) in the thalamus.



(A-C) Nell2 expression in the developing dorsal lateral geniculate nucleus (dLGN) was detected by RNA *in situ* hybridization in postnatal day (P) 4-12 mice (purple). (D) Retinal axons from the ipsilateral retina were labeled with an axon tracer dye (white). Nell2 is expressed in the domain of the dorsomedial dLGN where ipsilateral axons terminate.



Retinal axons of the right and left eyes were labeled at postnatal day (P) 9 by injection of cholera toxin B (CTB)-Alexa Fluor 488 (green) and CTB-Alexa Fluor 594 (magenta), respectively. The left dorsal lateral geniculate nucleus (dLGN) in coronal sections prepared at P12 are shown. In wild type, ipsilateral axons are confined to a single patch in the inner region of the dLGN, and contralateral axons terminate in the surrounding areas. In contrast, in Nell2 null (Nell2$^{-/-}$) mice contralateral axons invade the ipsilateral area (asterisks), forming a mosaic pattern of contralateral and ipsilateral axons. Dorsal is at the top and lateral is on the left.

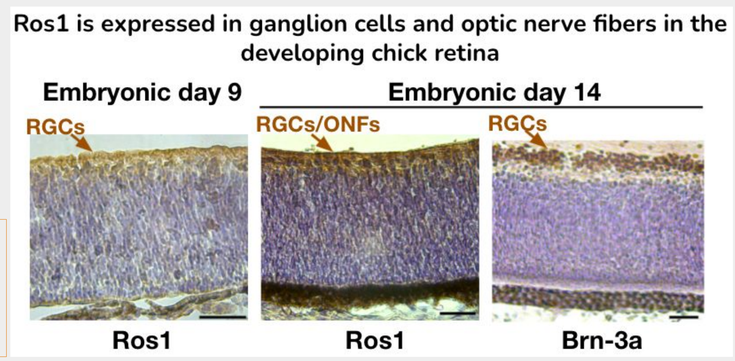


Explants of the ventrotemporal (containing ipsilaterally projecting retinal ganglion cells (RGCs)) (A,C) and ventronasal (containing contralaterally projecting RGCs) (B,D) retinae were prepared from day 15.5 mouse embryos and cultured for 72 h on a substratum coated with Nell2-AP (Fusion protein of N ell2 with an alkaline phosphatase tag) (A,B) or control AP (unconjugated alkaline phosphatase) (C,D), and axon outgrowth was quantified (E) (n=4 for each condition). Nell2-AP significantly inhibited outgrowth of ventronasal retinal axons.

METHODS

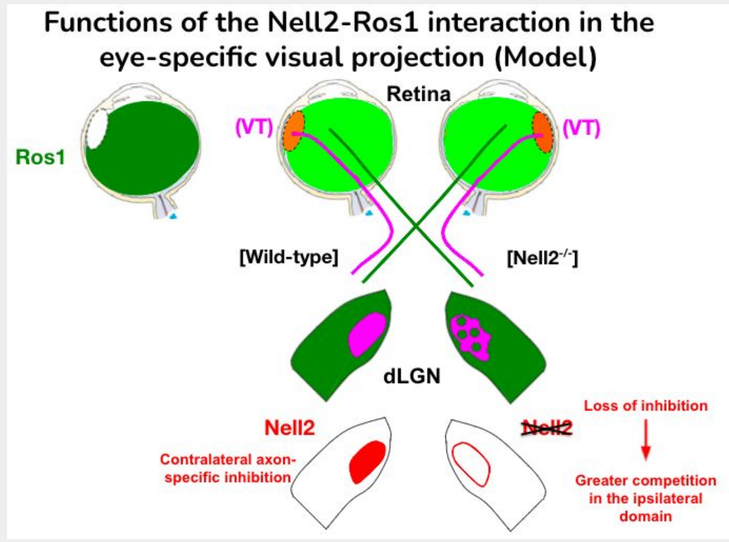
- Cryosectioning
  - Fix the dissected eyes with 4% paraformaldehyde at 4°C overnight
  - Cryoprotect with 30% sucrose at 4°C overnight
  - Embed the tissues in OCT compound and freeze
  - Cryosectioning at 10 µm
- Immunohistochemistry
  - Rinse the slides with PBS for 5 min x3
  - Antigen retrieval with 10 mM citrate (pH 6.0), 0.05% Tween-20
  - Treat with 3%H_2O_2 for 10 min, Rinse with PBS for 5 min x3
  - Blocking with 10% goat serum
  - Incubate with primary antibody for 1 hr. Rinse with PBS for 5 min x3
  - Incubate with secondary antibody for 1 hr. Rinse with PBS for 5 min x3.
  - Incubate with Elite ABC reagents for 30 min
  - Rinse with PBS for 5 min x3 and then with TBS for 5 min
  - Incubate with DAB for 30 min

RESULTS



Retinal sections were prepared from chick embryos at embryonic day (E) 9 and E14 and subjected to immunohistochemistry using anti-Ros1 and anti-Brn3a antibodies. Ros1 expression was detected in retinal ganglion cells (RGCs) (at E9 and E14) and in optic nerve fibers (ONFs) (at E14). Brn-3a is a marker of RGCs. The sections were counterstained with hematoxylin. Scale bars, 50 µm.

CONCLUSIONS



A model of the function of Nell2-Ros1 interaction in the eye-specific visual projection. Nell2 is expressed in the dorsomedial domain of the dorsal lateral geniculate nucleus (dLGN), where ipsilateral axons terminate (Ipsilateral domain). Ros1 is expressed in contralaterally projecting retinal ganglion cells. The Nell2-Ros1 interaction mediates a contralateral retinal axon-specific repulsion in the dLGN. Contralateral RGC axons are repelled by Nell2, and thus they avoid the ipsilateral domain and project to the surrounding contralateral domain. In contrast, ipsilateral axons can project to the ipsilateral domain. Ipsilateral axons do not terminate in the contralateral domain because there is greater axon-axon competition, thus they prefer to avoid this competition and arborize only in the ipsilateral domain. In Nell2−/− mice, the Nell2-mediated repulsion is removed, and contralateral axons can now invade the ipsilateral domain and compete more effectively with ipsilateral axons there. Subsets of the ipsilateral axons lose competition and spread out into surrounding areas in the contralateral domain.

FUTURE DIRECTIONS

In future studies, we will investigate the function of Ros1 in the eye-specific visual projection by using genetically modified mice in which the Ros1 gene is deleted (Ros1 knockout mice) and *in vitro* axon behavior assays. We will also study Ros1 functions in chick, where the Nell2-Ros1 may be involved in the layer-specific visual projection. We expect that our research will provide novel insights into the molecular mechanisms for development of binocular vision and neuronal network formation.

ACKNOWLEDGEMENTS

We would like to thank Valparaiso University and the Biology Department for the opportunity of the summer research project. The current project is supported by a grant from the Indiana Space Grant Consortium (INSGC)/NASA. Our previous work that led to this project was supported by research grants from Biotechnology and Biological Science Research Council (BBSRC, UK) and Royal Society (UK) to M.N.