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RPPR Final Report
as of 24-Jan-2019

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Major Goals: The objectives of the proposed research are to study the mechanisms and physiological roles of sensory integration in visual system functions. In all vertebrate species examined thus far, the retinas receive centrifugal input from the brain. In fish, the centrifugal pathway originates from olfactory terminalis neurons (TNs). The TNs project axons to the retina where they synapse with dopaminergic amacrine cells, and occasionally, retinal ganglion cells. In this research, we will study the mechanisms and roles of olfacto-retinal sensory integration in modulation of retinal sensitivity. The research will be conducted using transgenic zebrafish models. In Aim 1, we will test a hypothesis that in the retina the olfactory signals are separately processed by GnRH- and dopamine-mediated signaling pathways. In Aim 2, we will identify key factors that regulate TN axonal projection to the retina. The proposed research is not about fish vision or olfaction; instead it will study the fundamentals related to multi-sensory perception. The goal of this research is to understand the mechanisms of sensory integration in vertebrates including humans.

Accomplishments: During the funding period, we examined the development and pathfinding of terminalis nerves (TNs) in zebrafish. The TNs are located in the olfactory bulb, and they project axons to many brain areas including the neural retina. Using genetic and immunocytochemical techniques, we demonstrated that in the retina, the TN axons synapse with dopaminergic interplexiform cells (DA-IPCs). Furthermore, we examine the role of GnRH (released by TN) and dopamine (released by DA-IPC) signaling in TN axon projection to the retina using transgenic zebrafish. The research was conducted using both developing embryos and adult animals. The results from this research were published in Developmental Neuroscience. In summary, we accomplished the goals proposed in the research plan.

Training Opportunities: During the finding period, we trained three undergraduate students (Delaney Pfister, Dasom Kim, Audrey Drewing) and one graduate student (Jingling Li). All of them were from the Univ of Notre Dame. They have graduated between 2015 and 2017.

Results Dissemination: Nothing to Report

Honors and Awards: Nothing to Report

Protocol Activity Status:

Technology Transfer: Nothing to Report

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Article Title: Zebrafish olfacto-retinal centrifugal axonal projection: Effects of dopamine and GnRH signaling
Authors: Delaney Pfister, Chuanjiang Yu, Dasom Kim, Jingling Li, Audrey Drewing, Lei Li
Keywords: Terminalis nerve, axon projection, GnRH, dopamine, retinal, zebrafish
Abstract: In zebrafish, the TNs are located in the olfactory bulb. The TNs project axons to many brain areas, which include the neural retina. In the retina, the TN axons synapse with dopaminergic interplexiform cells (DA-IPCs) and retinal ganglion cells (RGCs). In this research, we examine the role of GnRH and dopaminergic signaling in TN axon projection to the retina using the transgenic zebrafish Tg(GnRH-3::GFP). While the TNs developed at 34 hours post-fertilization (hpf), the first TN axons were not detected in the retina until 48-50 hpf, when the first DA-IPCs were differentiated. In developing embryos, inhibition of retinal GnRH signaling pathways blocked the projection of TN axons to the retina. However, inhibition of retinal dopaminergic signaling produced little effect on TN axon projection. In adult retinas, inactivation of GnRH receptors disrupted the patterns of TN axon distribution, and depletion of DA-IPCs abolished the TN axons. When DA-IPCs regenerated, the TN axons reappeared.
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Acknowledged Federal Support: **Y**

RESEARCH SUMMARY

The goal of the proposed research is to investigate the mechanisms and developmental courses of terminalis nerve (TN) input in retinal functions using zebrafish models. In according to the research proposal, during the funding period, we performed different sets of experiments. We accomplished the goals proposed in our research plan. Below are summary of the experiments we performed. Some of the research results have been published.

Determine the effects of dopaminergic signaling on TN axonal projection

We examined the role of dopamine signaling in axon pathfinding during embryonic development. The research was conducted using transgenic zebrafish (developing embryos and adults), in combination with the use of immunocytochemical methods. We found that dopamine may function as attractive fact that guides the projection of TN axons to the retina (Fig 1, Fig 2, and Fig. 3).

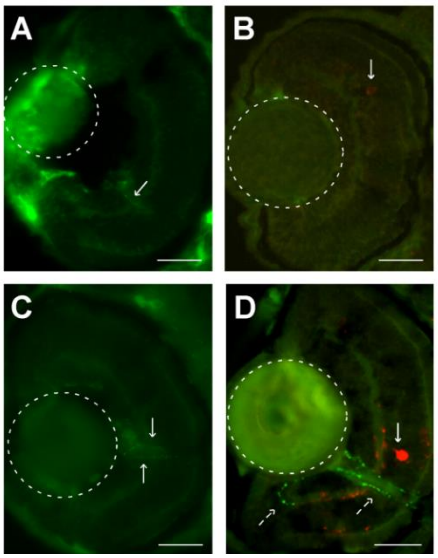

Fig 1. Cryostat sections of transgenic Tg(GnRH3::GFP) fish that show the TN axons (detected by the expression of the GFP conjugated transgene) and dopaminergic cells (labeled by anti-TH antibodies). A, B: Retinal sections from embryos between 48 and 50 hours of age. TN axons were observed in the optic nerve (A, arrow). At the same developmental stages, a dopaminergic cell was seen in the inner nuclear layer (B, red, pointed by the arrow). C: A cross section from a 52-hour-old embryo. Branches of TN axons were seen in the optic nerve (arrows). D: A cross section from a 62-hour-old embryo. Note that the TN axons (green, pointed by arrows with dashed lines) elongate toward to dopaminergic cell (red, pointed by the arrow with solid line). Dashed circles outline the lens. Scale bar: 50 μm.

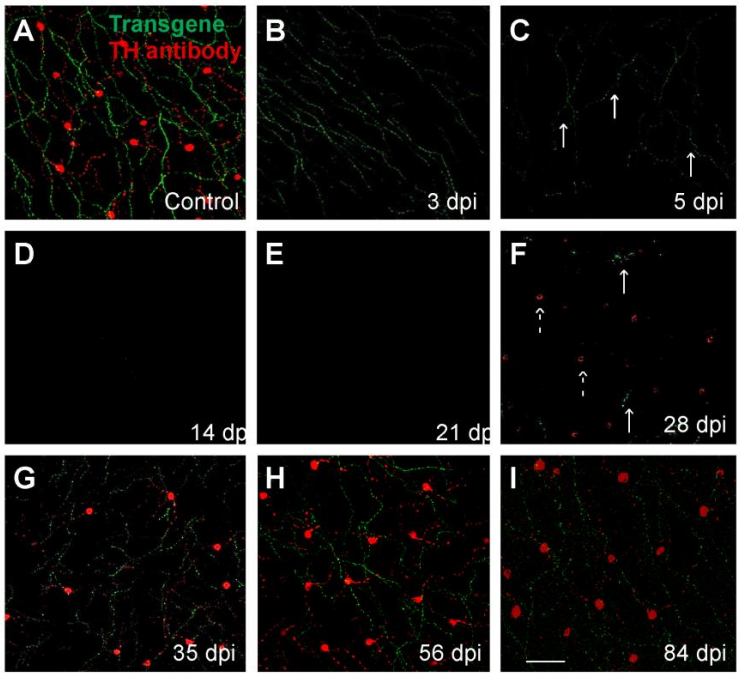

Fig 2. Whole-mount retinas of adult Tg(GnRH3::GFP) fish that show the TN axons and dopaminergic cells before (A) and after 6-OHDA treatment (B-I). Images were taken in the nasal-ventral retina, adjacent to the optic nerve. Note the lack of TH immunoreactivity at 3, 5, 14 and 21 dpi (days post injection; panels B, C, D and E). On 28 dpi, regeneration of dopaminergic cells was evident (red, pointed by arrows with dashed lines) and re-distribution of the TN axons (green, pointed by arrows with solid lines) in the retina was detected. At 35, 56 and 84 dpi, abundance in dopaminergic cells and TN axons were found in the retina. Scale bar: 50 μm.

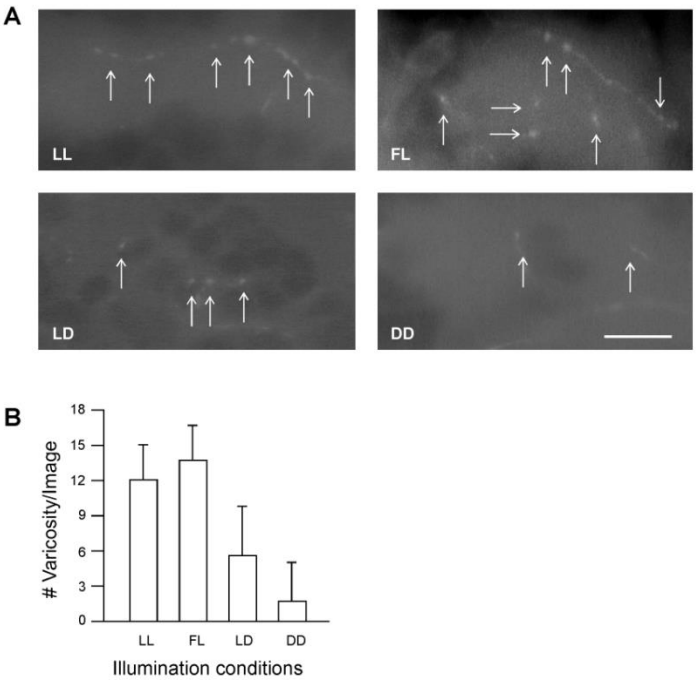

Fig 3. Effects of different lighting conditions (which result in different concentrations of dopamine in the retina) on TN axonal projection. A: Fluorescent images of 7-day-old retinas grew up under different lighting conditions. The TN axons were labeled by the GnRH::GFP transgene. Images were taken in the ventral retina adjacent to the optic nerve (captured using a regular camera, not confocal). LL, constant light; FL, randomly expose to 15-min light pulses during the day and night; LD, normal light-dark cycles; DD, constant dark. Scale bar: 100 μm. B: Quantitative analysis of TN axonal varicosities in the retina under different lighting conditions. Data represent the means ± SE. (n=8 for each condition).

Determine the expression of GnRH receptors in zebrafish retinas

We examined the expression of GnRH receptors in zebrafish retinas using whole-retina and single-cell PCR as well as in situ hybridization methods. We found that the GnRH receptors are expressed in the retina, where the mediate the development and function of TN input from the olfactory bulb (Fig 4 and Fig 5).

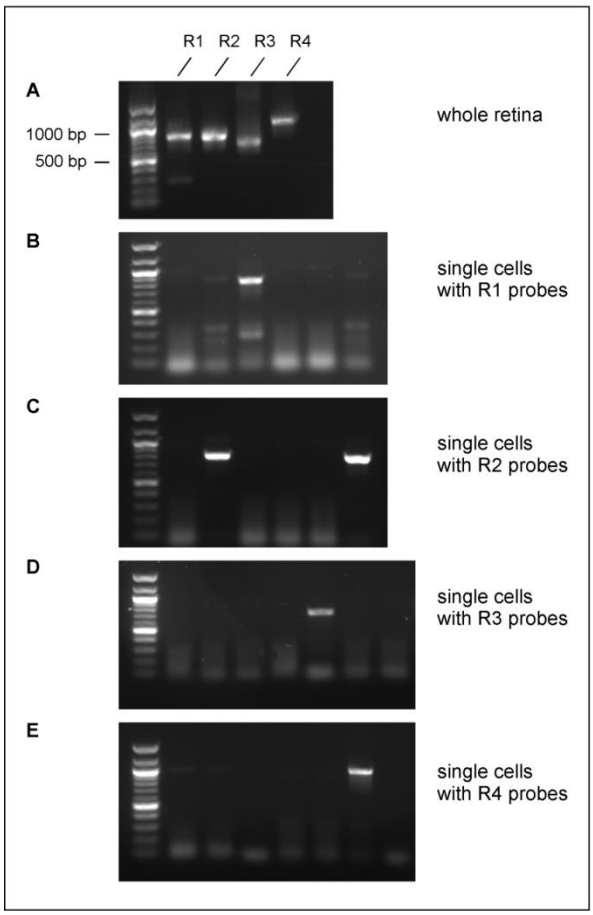

Fig 4. RT-PCR of the expression of GnRH receptors in zebrafish retinas. **(**A) Whole-retina RT-PCR. The PCR products of GnRH receptors (R1-R4) are 831, 807, 676, and 1,004 bp, respectively. We detected the expression of all the 4 types of GnRH receptors fronm whole-retina preparations. (B-E) Single-cell RT-PCR with R1, R2, R3, and R4 receptor probes, respectively. Cells were isolated in dissociated retinal tissues. Based on their morphologies, they are most likely amacrine cells and ganglion cells. However, we haven't identified the cell types using cell-specific gene markers. Note that individual GnRH receptor types were detected in individual retinal cell types.

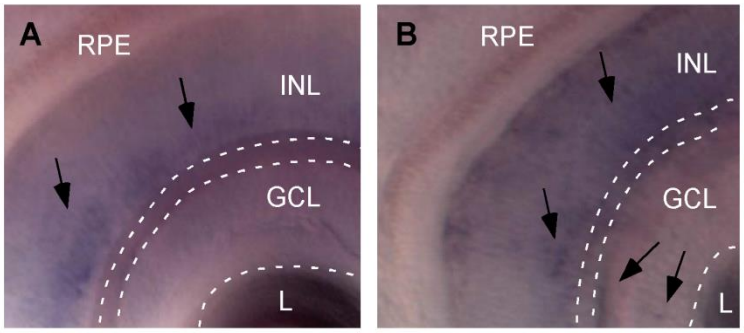

Fig 5. In situ hybridization of type-1 (A) and type-2 (B) GnRH receptors in zebrafish retinas (from 5 dpf embryos). It showed that type-1 receptors are expressed in the INL, whereas type-2 receptors are expressed in the INL and GCL (arrows). Dashed lines outline the lens and interplexiform layer. Abbreviations: RPE, retinal pigment epithelia; INL: inner nuclear layer; GCL, ganglion cell layer; L, lens.

Determine the effect of GnRH signaling in TN axonal pathfinding

We also examined the effects of GnRH signaling transduction on TN projection in developing zebrafish retinas. Because the retinal cells express GnRH receptors, we tested a hypothesis that GnRH, which is released in from the TN, is required for TN axonal projection to the retina. The experiments were performed using transgenic zebrafish, and the function GnRH receptors were blocked by intraocular injection of pharmacological compounds MPDM. We found that in the lack of GnRH signaling, TN axons is no longer projected to the retina (Fig 6).

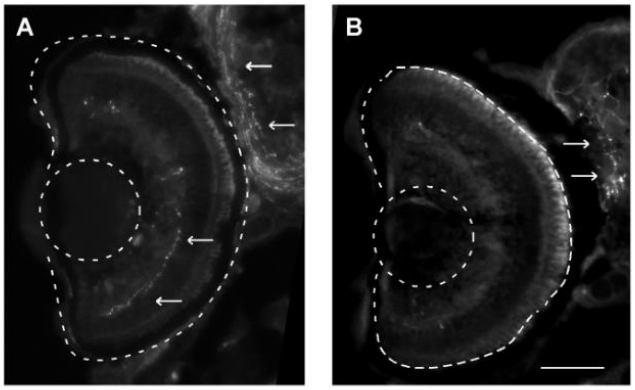

Fig 6. Cryostat sections that show the TN axons in control (A) and MPDM-injected (B) animals. In control animals, TN axons (labeled by the expression of the transgene GnRH3::GFP; pointed by arrows) were seen in the retina and the brain. However, in MPDM-injected fish, no GFP-labeled TN axons were detected in the retina, but TN axons were seen in the brain (arrows). Dashed lines outline the retinas and lenses. Scale bar: 50 μm.