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The Effect of Semaphorin 3A on Chick Embryo Retinal Growth Cones

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The Effect of Axon Guidance Molecule Semaphorin 3A on Different Aged Chick Retinal Ganglion Cells

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Abstract

During embryonic development, axons grow from the retina of the eye to the tectum of the brain which allows for visual information transfer. Axons travel to the tectum via axon pathfinding, which is influenced by axon guidance cues, such as our area of interest: Semaphorin 3A (Sema 3A). Axon guidance molecules interact with retinal ganglion cells (RGCs), which contain growth cones- a crucial feature of the developing visual system. Growth cones are extensions of growing or regenerating axons supported by microfilaments growing to their synaptic target, in this case the brain. Some inhibitory axon guidance molecules are known to cause growth cone collapse and Sema 3A is one of them. When growth cones collapse, they cease growth and then retract, never making it to their target. While Sema 3A's importance is known in the nervous system, a study conducted by Luo et al. (1993) demonstrated that Sema 3A causes growth cone collapse of chick embryo dorsal root ganglion cells (DRGs) but not growth cone collapse in chick retinal ganglion cells (RGCs). We have found significant evidence that Sema 3A does indeed cause growth cone collapse when it comes in contact with embryonic chick retinal ganglion cells, which is inconsistent with the Luo et al.(1993) finding. After further investigation of this experiment, we have found that RGC's have the ability to regenerate from collapse after a 15-20 minute time window, thus giving the illusion to Luo et at. (1993) that Sema 3A does not affect RGC's because of the 60 minute allotted time window they used. We are currently investigating the effect of Sema 3A on different embryonic ages (E4, E5, E67, E8). We have found preliminary data that suggests that Sema 3A will give the same effect on RGC's no matter the age.

Introduction

In a developing visual system axon guidance molecules are crucial for guiding axons to their synaptic targets. Semaphorin 3A is an axon guidance cue in the nervous system that contributes to developing the visual system. Axon guidance molecules are known for their interaction with growth cones. Growth cones are actin-supported extensions of a developing or regenerating axon looking for its synaptic target. In order for the growth cone to reach the proper target, coordination between microtubules and filamentous actin is required (Dent et al. 2011). Throughout development, communication between environmental signaling and the cytoskeleton takes place at the neuronal growth cone (Dent et al. 2011). When growth cones collapse, filopodia on the ends of the growth cone collapse, meaning they halt in movement, and they retract and grow in a different direction after regeneration. Numerous signaling pathways are involved in the process when a growth cone collapses (Dent et al. 2011). Some inhibitory axon guidance molecules are known to cause growth cone collapse and Sema 3A is one of them. While Sema 3A's importance is known in the nervous system, a study conducted by Luo et al. (1993) demonstrated that Sema 3A causes growth cone collapse on chick embryo dorsal root ganglion cells (DRGs) but not growth cone collapse in chick retinal ganglion cells (RGCs). This absence in Sema 3A sensitivity in RGCs gave rise to the belief that Sema 3A is not an important axon guidance molecule in the visual system. However, we found that Sema 3A does cause growth cone collapse in chick embryo ganglion cells, which is inconsistent with the Luo et al. (1993) finding.

Materials and Methods

- Chicken eggs were incubated for six days at 39°C in a humidified rocking incubator.
- The E6 chick embryo (see fig 5) retina was dissected and cut into small pieces called explants. E5, E7, and E8 were also used for the age analysis.
- The explants were plated onto PDL and Laminin coated dishes with Ham's F12 media containing N2, B27, Penicillin/streptomycin, L-glutamine, Ascorbic acid, sodium pyruvate, and growth factors BDNF and CNTF
- The explants were cultured overnight at 37 °C and 5% CO2.
- The explants were treated with different concentration of Sema-3A supernatant from transfected HEK293T, 1:100, 1:1000 and 1:1000 for different time periods. The control was 1:100 from untransfected HEK293T cells.
- The cells were then fixed with 4% paraformaldehyde in 0.1M of phosphate buffer pH 7.4
- The cells were permeabilized with 0.1% Triton X-100 in 1X PBS and stained with Alexa Fluor 488 Phalloidin overnight.
- The retinal explants were examined for normal or collapsed growth cones using a fluorescence microscope (see fig. 1 and fig. 2)
- The growth cone collapse rate was quantified and statistics with Fisher's exact test.

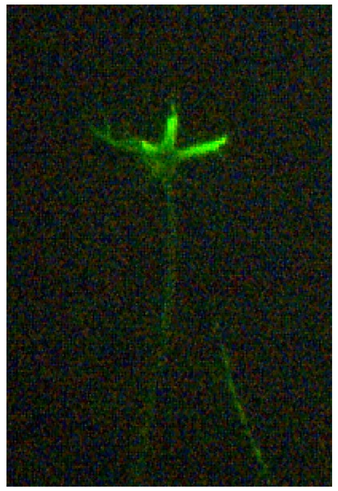

Figure 1: A normal growth cone in a 1:100 Sema 3A dilution.

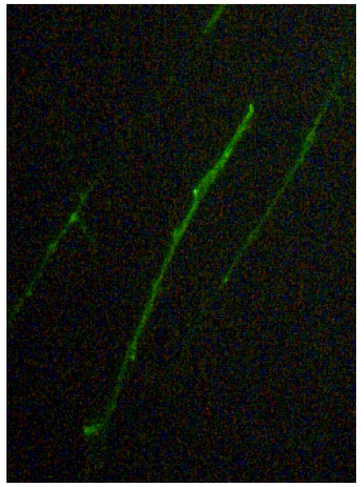

Figure 2: A collapsed growth cone in a 1:10000 Sema 3A dilution.

Results

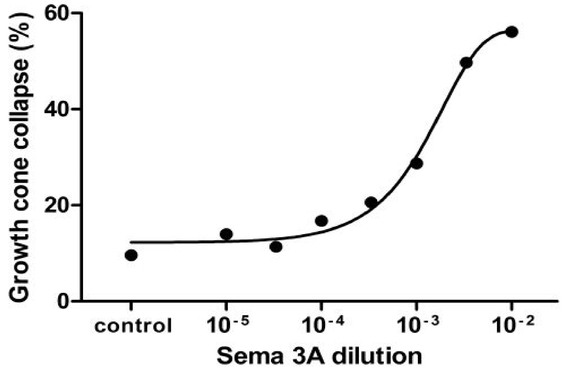

Figure 3: The graph shows a dosage response curve between Sema3A and E6 growth cone collapse. As the Sema3A concentrations increase, growth cone collapse increases and then levels off. Data from Maja Stefanowska-Cieslak.

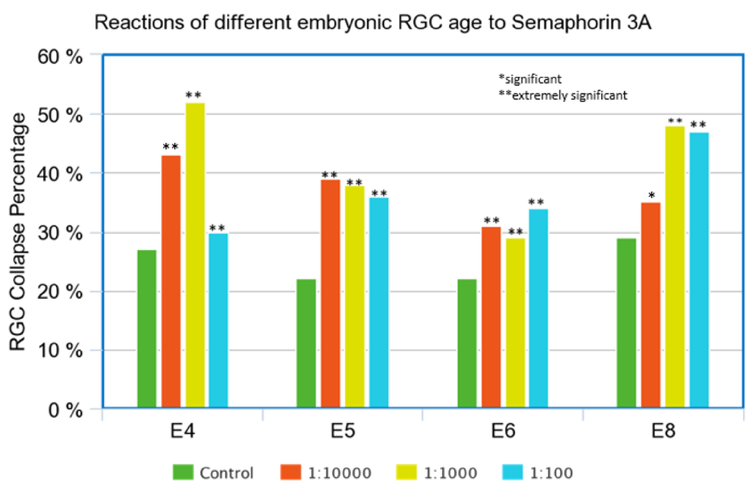

Figure 4: This graph shows preliminary data of three dilutions of Sema 3A with control, on four different ages of chick embryo.

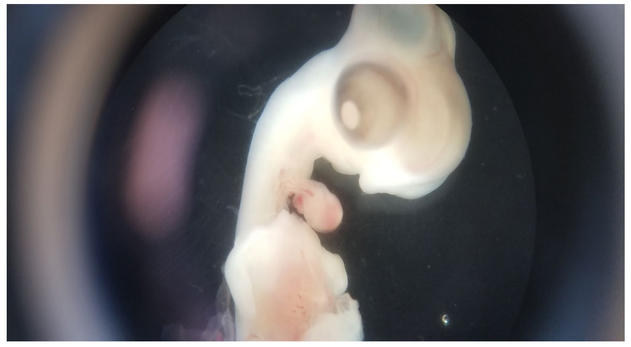

Figure 5: Picture of an E6 chick embryo

Discussion

This investigation involving different ages was sparked by a study conducted by Campbell et al. (2001). They found that different ages of *Xenopus* RGC's will react differently to the same treatment of Sema 3A. Stages 35/36 and 37/38 growth cones show significant increases in collapse compared with stages 24 and 28 (Campbell et al. 2001). Though chick and *Xenopus* visual systems are different, both are driven by axon guidance. As we continue to investigate, we hope to uncover more answers about axon guidance in the developing chick visual system.

As shown in Figure 3, we can see a dose response curve when E6 chick retinal ganglion cells are treated with Semaphorin 3A. We used this data to compare the results from different aged chick RGCs. Comparing to Stefanowska-Cieslak's data, we examined a dose response curve for in each age. From our preliminary data, we can still see somewhat of a dose response in each age group as shown in Figure 4. A statistical analysis was conducted within each age group, comparing each dilution to its respective control. All, except for one test, came back as extremely significant (**) with $*p < 0.0001$. However, this data is preliminary, and more data is being collected. With more data collected, we hope to see a clearer dose response curve and determine if different ages respond to the Sema 3A treatment differently.

Works Cited

Campbell DS, Regan AG, Lopez JS, Tannahill D, Harris WA, Holt CE. 2001. Semaphorin 3A elicits stage-dependent collapse turning and branching in Xenopus retinal growth cones. J Neurosci. 21(21):8538-8547.

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Acknowledgments

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