**Synesthesia** is a neurological condition in which perception of a physical stimulus, such as a written letter, triggers an automatic and unrelated sensory response, like the color yellow. Many different forms have been documented [3]. This disorder is currently diagnosed using memory-based assessments of associations, as synesthetes display enhanced learning and memory retention [5]. While the specific mechanism behind synesthesia is not known, these experiences have been attributed to overlapping sensory networks [4]. *ROBO3* – a regulatory gene in the axon guidance pathway which directs neuronal network development – has been found to be mutated in people with sound-color synesthesia. *However, it is not currently understood how the role of ROBO3 in the process of axon guidance relates to enhanced learning through color perception.*

The **objective** of this project is to identify specific ROBO3 mutations that cause aberrant axon development which impact color perception and learning. I **hypothesize** that ROBO3 mutants will be able to learn information received through color perception more quickly than unmutated model organisms. This hypothesis is supported by research exploring the role of ROBO3 mutations in *Drosophila* photoreceptor development, and by the human phenotype of increased memory efficiency of color-associated recall for synesthetes [1, 5]. I chose zebrafish as my **model organism** because of their transparent nervous systems, ability to perceive color, and prior use in learning studies. My **long-term goal** is to analyze how color perception impacts learning and memory development for diverse species.

**Aim 1: Identify specific protein domain regions within ROBO3 that contribute to color perception.**

Approach:NCBI BLAST will be used to find ROBO3 homologs in diverse species with varied color perception and visual systems. Pfam will identify protein domains conserved within ROBO3 homologs. Domain sequences will be aligned, and phylogenetic trees will be built with MEGA-X for each domain type. Analyzing clades for similarity in visual system organization and color perception, SNPs of interest can be identified. Domain SNPs will be introduced into zebrafish models using CRISPR/Cas9, and groups of fish will then be sent through a color-driven maze assay and timed upon repeated attempts in comparison with control wildtype (WT) zebrafish [2].

Rationale:Identifying protein domains of ROBO3 strongly correlated with color perception will help develop candidate mutations to be studied in further assays.

Hypothesis:Protein domain regions in ROBO3 that are highly conserved among groupings of organisms with more complex visual systems will be found to be significant in color perception.

**Aim 2: Identify genes that are differentially expressed in ROBO3 mutants.**

Approach:Visual cortex, hippocampus, and medial entorhinal cortex brain tissues samples will be taken from naïve WT and ROBO3 mutant zebrafish and from WT and mutants after completing the color maze assay multiple times. RNA-sequencing will be conducted on these tissue samples and GO analysis will sort differentially expressed genes into categories like spatial learning, visual perception, and sensory perception of light stimulus.

Rationale:Identifying differentially expressed genes will elucidate the gene interactions that confer enhanced learning in ROBO3 mutants.

Hypothesis:WT and ROBO3 zebrafish pre-maze will differ in gene expression related to color perception, but WT and ROBO3 mutant maze-tested zebrafish will differ in gene expression related to spatial learning, memory formation, and cognition.

**Aim 3: Quantify protein expression involved in color perception and memory formation.**

Approach:Using SILAC in the four zebrafish treatment groups above, protein expression within the brain can be quantified. The proteins found to be differentially regulated only in the ROBO3 mutants that have completed the maze will be grouped by their GO terms to better understand biological and functional relationships with ROBO3.

Rationale:Understanding how ROBO3 protein interactions change in the brain after mutation and after learning through color perception will further explore the biological processes behind enhanced learning in synesthetes.

Hypothesis:Proteins implicated in color perception and memory formation (learning) will be upregulated in maze-run ROBO3 mutants.

Through these aims, I expect to better understand how ROBO3 mutations in color-perceptive synesthetes correlate to enhanced learning and memory formation. This is impactful research because understanding the biological role of color perception in these processes can be utilized to positively affect the way educators and communicators teach difficult concepts and allow individuals to develop enhanced learning skills through color associations.

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