Abstract
The presence of the neurotransmitter GABA in the mammalian inner ear is well established, yet its role in regulating inner ear cell function is less clear. We seek to understand the role of GABA in the inner ear by using the model organism zebrafish. Zebrafish possess a sensory system that has been dissected from the rest of the body of the fish into the environment. These so-called hair cells are remarkably similar to the sensory cells of the cochlear and semicircular canals. Because they are on the outside of zebrafish, and not behind a bony skull, lateral line hair cells are easily accessible for study. Therefore, we are determining if we can use the lateral line system to understand more about GABA in the inner ear. We have used RNA extraction and RT-PCR to detect the expression of 27 GABA-related genes in zebrafish.

Introduction
The Transmitter GABA

- GABA is released by neurons and binds to either presynaptic or postsynaptic GABA Receptor (GABAR) proteins.

- GABARs: Ion channels formed from 20 possible subunit isoforms.

- GABARs: G protein-coupled receptors formed from 3 possible subunit isoforms. Accessory KCTD proteins associate with GABARs.

- GABA, and multiple isoforms of GABARs and GABARs, have been detected in the mammalian inner ear (1-2).

- The first physiological role for GABARs was recently determined: researchers showed that GABA acts as an autoinhibitory signal on presynaptic efferent terminals via GABARs, and not GABARs (3).

- The physiological role of GABA and GABARs in the inner ear thus remains poorly understood.

The Zebrafish Lateral Line

- The lateral line is used by aquatic vertebrates to detect water movement.

- It is closely related to the inner ear, and is externally located and therefore more easily accessible for study.

- We seek to use the zebrafish lateral line as a model to study the function of GABA in the mammalian inner ear.

- Here, we have investigated whether the GABAR isoforms expressed in zebrafish correspond to the mammalian orthologs that have been detected by others (1, 2, 3).

- We have isolated zebrafish tissues, including larval head, trunk and adult skin. Lateral line tissue remains associated with dissected skin, and we therefore hypothesized that the skin sample would serve as a proxy for isolated lateral line.

- We extracted RNAs, synthesized cDNAs, and amplified regions of all 20 GABA R, 3 GABA Rs, and 6 KCTD accessory protein isoforms, as well as 3 isoforms of the enzyme necessary for GABA synthesis, GAD.

Results

- **GAD, GABA R α, and GABA R isoforms**

- **GABA R β and γ isoforms**

- **GABA R ρ, δ, ζ and n isoforms**

- **Novel Alternative Exon in GABA R p2b Gene**

- **Alignment of Gel-extracted PCR Products**

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Figure 1. Tissue-specific expression of GABA-related genes in zebrafish. 1% agarose gels showing products from GABA-related gene amplifications. RT-PCR were extracted from zebrafish tissues, as indicated at left of gels using RNaseasy Mini Kit (Qagen). RT-PCR was performed on genes identified with ZFIN and NCBI, using gene-specific primers designed with NCBI Primer BLAST. Supernatant from First-Strand Synthesis System (Invitrogen), and GoTag Hot Start PCR Master Mix (Promega). Target genes are indicated above gels, and those that are candidate lateral line genes are highlighted in purple. S: products confirmed by sequencing; OT: off- target product discovered by sequencing. Select size markers labeled at left in basepairs, gapdh is an RT-PCR control gene, and cdh2 is a hair-cell specific gene and is thus a control for hair-cell RNAs in tissue samples.

Figure 2. Novel alternative exon discovered in GABA R p2b gene. Two PCR products from amplified cDNAs were gel extracted and sequenced. Sequencing revealed a cassette exon, exon 3 (e3), which is included or not in final mRNAs. Amino acid sequence of closely related isoforms in all zebrafish p isoforms reveals a high degree of sequence conservation.

Figure 3: Candidate genes: GABAR and GAD genes identified in zebrafish skin, GABAR and GAD subunit isoforms were, or were not, detected in zebrafish skin by RT-PCR, gel electrophoresis and sequencing. Isoforms labeled in red were not detected, and thus have been eliminated as candidate isoforms. Isoforms labeled in purple were found in adult skin, and thus remain candidate lateral line GABAR genes.

Conclusions + Future Directions
We detected 31/32 GABA-related genes in whole zebrafish larvae.

GABA Rs dimers expressed in zebrafish skin (GB1a + GB2) are the same as those found in mammalian inner ear (3), suggesting a presynaptic GABA R location.

GABA R isoforms detected in zebrafish skin include some isoforms detected in mammalian inner ear (1, 2), but not all.

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References