A pain to stain. Trying to elucidate gene expression $GABA_A$ receptor subunits α_1 and β_2 in the brain and retina of adult zebrafish via *In situ* hybridization

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My study focused on the gene expression of two genes which code for different subunits of the $GABA_A$ neurotransmitter receptor. This neurotransmitter is one of the most prominent inhibitory neurotransmitter in the central nervous system. It associates with benzodiazepine, barbiturates, ethanol, and general anesthetics. The arrangement and composition of the $GABA_A$ neurotransmitter affects its pharmacological properties. Five subunits, which may arise from 8 gene families ($\alpha_{1\text{-}6b}$, $\beta_{1\text{-}4}$, $\gamma_{1\text{-}3}$, δ, ε, π, $\rho_{1\text{-}3a,}$ ζ), contribute to forming the neurotransmitter. We used the zebrafish, *Danio rerio*, as a study model for it is one of the most widely utilized study specimen in the field of behavioral neurobiology. A previous study showed that the α_1 and β_2 subunit is among the most highly expressed subunits in the adult zebrafish brain and retinas. Therefore, I investigated the distribution patterns of these subunits using *in-situ* hybridization. This procedure uses DIG-labeled RNA probes to hybridize with the mRNA in the fixed brain or retinal tissue. After hybridization, we are able to visualize the distribution by color detection produced by alkaline phosphatase activity. Distinct, targeted staining results in a deep purple color whereas unspecific staining is a pinkish hue. Unspecific staining or background staining occurs when the probe hybridizes with non-targeted RNA. We reduced the background staining by optimizes the specificity of the probe and removing non-targeted RNA. In order to be sure the staining observed is not just by happenstance, we also used a control probe. The control probe was created with sense DNA strand which does not produce protein coding DNA. Therefore, staining is only specific if the control probe does not show the same staining patterns as the experimental probe.

So what did we find? In the brain, the α_1 subunit was found in the dorsal and ventral telencephalic area and the preglomerular nucleus. The β_2 subunit was seen in the periventricular gray zone, torus longitudinalis and immediate area surrounding the vulva cerebelli, corpus mamillare, lateral recess, and areas of the caudal zone of periventricular hypothalamus. In the retinas, the α_1 and β_2 antisense probe showed specific staining in the outer nuclear layer and inner nuclear layer. The α_1 sense probe did not show staining in the brain or retina, but the β_2 probe did. Therefore, the β_2 probe staining cannot be trusted.

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