Using GFP-Aequorin to study the role of serotonergic neurons in the dorsal raphe nuclei of larval zebrafish

Abstract

The neurotransmitter serotonin (5-HT) has been implicated to modulate numerous physiological processes and behaviors, such as appetite, aggression, and anxiety. However, the functional pathways underlying the numerous outputs of serotonin activity are still not well understood. My study aimed to deepen the understanding of the role of serotonergic neurons in larval zebrafish (4-7dpf) and focused primarily on the prominent serotonergic neurons in the superior and inferior raphe nuclei within the hindbrain. To investigate the activity of these neurons, a plasmid with the promoter petl to drive the expression of GFP-apoAequorin (Ga) exclusively in the neurons within the raphe nuclei of larval zebrafish was first constructed. When complemented with its substrate coelenterazine, the bioluminescent GFP-Aequorin (GA) can reveal the depolarization of the targeted neurons by emitting green photons triggered by an elevated calcium concentration. Following microinjection of plasmid DNA, zebrafish larvae (3dpf) with transient expression were then exposed to coelenterazine for 24-48 hrs. Simultaneous monitoring of green photons emitted exclusively by petl:Ga-labeled neurons and motion of the fish revealed serotonergic neuronal activity in freely swimming zebrafish at 5-6dpf. Behavioral assays were utilized to analyze the activity of the serotonin neurons under several different conditions. Furthermore, about 1 00 zebrafish with transient expression of pet1:Ga were raised to generate a stable transgenic line for use in future studies. Thus a powerful tool was developed for further investigating and elucidating the role of serotonin neurons in freely behaving zebrafish.