AUTOMATED QUANTIFICATION OF ANXIETY AND SOCIAL BEHAVIORS IN FREE-SWIMMING LARVAL ZEBRAFISH

James B. Newton (Adam Douglass, Ph.D.)

Department of Bioengineering

Oxytocin (OXT), a neuromodulatory peptide produced by the hypothalamus, is involved in a variety of physiological and behavioral phenomena. Exogenous OXT and drugs that mimic OXT signaling may be potential treatments for autism, schizophrenia, and other neurological disorders. The canonical mechanisms by which OXT exerts its effects are neuroendocrine in nature, as the peptide is released into circulation through the neurohypophysis. Recently, however, some attempts have been made at characterizing the involvement of central nervous system circuitry in the peptide's influence on behavior. Though still poorly mapped, it is becoming increasingly apparent that centrally projecting OXT neurons do exist and directly modulate behavior via CNS targets. Larval zebrafish, due to their small size and clear skin, provide a prime model for the imaging and eventual mapping of central targets of OXT throughout the entire vertebrate brain. Detailed imaging of wild type and OXT-ablated larvae, coupled with behavioral analysis, may make possible a more complete understanding of these OXT neurons, linking neuronal maps to behavior.

This particular project better enables our broader goals with respect to OXT by providing the necessary behavioral analysis tools for linking OXT neurons to behavior. We have developed a means of robustly tracking and quantifying two especially relevant behaviors in the study of OXT—namely anxiety and social behaviors. Utilizing MATLAB image processing software, custom programs have been created which allow precise measurements of shoaling and thigmotaxis ("wall hugging"), corresponding to social and anxietal behaviors, respectively. Aerial images of free-swimming fish are taken at a given sampling frequency. A method of image subtraction is used for fish identification and tracking, while computational analysis provides quantification of each desired parameter.

The fish identification program works with high precision. The automated results of six trials (n=6) were compared to those provided by a manual method of fish identification. The average distance between the automated outputs for fish location and the corresponding hand-selected points differed by an average of .0137 mm, or less than ⅙ of a pixel. When both the hand-scored and automatically derived datasets were subjected to our shoaling and wall-hugging algorithms, the outputs differed in magnitude by an average of 0.79±0.64 mm and 0.89±0.69 mm respectively. Such precise quantification of anxiety and social behavior will allow for characterization of larval zebrafish behaviors directly influenced by OXT, thus contributing to a fuller understanding of the neuromodulatory peptide's neuronal mechanisms.