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Detecting startle responses in the zebrafish using novel digital imaging techniques

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The startle (escape) response has been measured in fish using various methods, including dropping an object into a fish tank, presenting an acceleration or acoustic tone or placing a fish in a drum with a spinning dark stripe. Each technique has its own complications; a dropped object may not have a controlled trajectory, the response could be mediated by visual changes, pressure changes, or a combination of both. Due to high visual responsiveness in the zebrafish, auditory tests must strictly control visual stimulation. This project's goal was to develop a computer vision-based tracking system to reliably and reproducibly measure startle responses in zebrafish. Startle response of zebrafish to auditory and visual stimuli were investigated. Near-infrared illumination (NIR) was presented from underneath the tank, & high-speed NIR video (100 frames per second) was recorded from an overhead view. The illumination of the tank was controlled by placing it inside a large enclosure; visible light was either provided via a florescent bulb or absent. The swimming distance of the fish was measured between each frame. For auditory testing, an underwater speaker was placed inside the tank, separated from the zebrafish by mesh & a 100 millisecond (ms) burst of 130 dB white noise (as measured in air) was presented. No change in baseline swimming distance could be detected when the auditory stimulus was applied, either under visible light or with the surroundings of the tank darkened. To test responses to a visual stimulus, a computer screen was placed next to the tank. The screen was either completely white (no stimulus), or displayed a randomly-located black circle on a white background, presented for approximately 1 second. The circle diameter was approx. twice the length of the fish. Presentation of the visual stimulus resulted in marked increases in startle response, as measured by travel distance. Typically the distance traveled was approx. 6 times the baseline distance traveled by the fish. This occurred for approx. 340 ms. This response was contingent on whether the fish was oriented toward the screen at the time of the presentation. In conclusion, digital imaging is a powerful method for accurately monitoring zebrafish behavior using cameras with a high temporal resolution. This approach can be used to monitor unconditioned responses to various stimuli and has potential application in phenotypic screening for behavioral genetics.

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