Behavioral Paradigms for Studying Neural Circuits of Social Behavior and Fear Conditioning in a Zebrafish Model of Autism

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Abstract

Zebrafish is an important genetically tractable vertebrate model system to study the development and function of brain circuits relevant to human affective disorders, like schizophrenia and fear and anxiety disorders. Our group uses zebrafish to study neural circuits and developmental factors underlying autism spectrum disorders. Patients with autism show compromised social behavior, deficiencies in smell and taste, and elevated anxiety and stress responses. Many of these behavioral abnormalities have been correlated with dysfunctions of the amygdaloid complex, which is the regulatory core of the emotional brain in humans and other vertebrates. To study how mutations of autism genes such as *shank3* affect the emotional brain and specifically the amygdala, we plan to perform behavioral experiments with a zebrafish shank3 mutant model of autism in comparison to normal (wild type) fish. Here, we present our research strategy and two behavioral experimental designs (paradigms) that will allow us to analyze the social and anxiety behavior of adult wild type zebrafish versus the shank3-mutant model of autism. Specifically, we are currently implementing a Zantiks behavioral chamber to apply mild electroshocks to the Zebrafish in conjunction with light stimulus for fear conditioning. In addition, we are setting up a social (shoaling) behavioral paradigm that allows us to track the movement and behavior of a single fish in a group of fish. The establishment of these behavioral paradigms will greatly help in studying the neural circuits underlying autism spectrum disorders.

Background

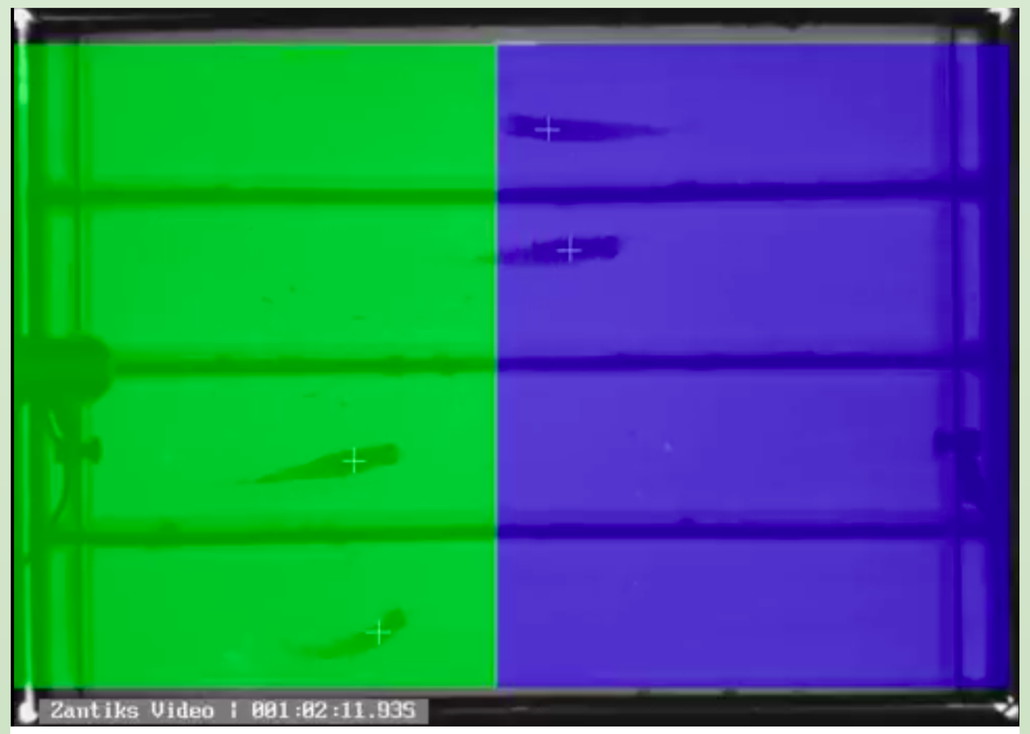
Zebrafish have become an increasingly important comparative model in neuroscience. Our lab utilizes this model in order to perform a variety of experiments. The *shank3a;shank3b* double-homozygous mutant model of autism will be compared to a wildtype sibling zebrafish in order to identify neurological differences. We use a Zantiks behavioral chamber in order to carry out fear conditioning experiments. The set up includes a tank with four separate lanes to house four fish, an LED screen to send visual stimuli and electroplates in the tank to send electrical currents.

Methods

Fear conditioning paradigms have been set up with many models. Our paradigm will pair a red light (CS, conditioned stimulus) with an electro shock (US, unconditioned stimulus). The conditioned stimulus will be a change of color on the LED screen, while the unconditioned stimulus will be aversive stimuli that evokes fear response like erratic movement and freezing. The fish will presumably develop a fear/anxiety of the visual change in relation to the aversive stimulus. In order to develop the model of fear in the brain, we will show a visual stimuli on the LED screen with sending small electroshocks to the zebrafish while in the Zantiks Behavior chamber. This will induce a state of fear and allow us to compare the neurological reactions between the *shank3*/wildtype.

Figure 1. Zantiks Chamber

Figure 2. Video capture



This is a still image from a Zantiks video. We will use tracking software in order to create figures of the movement.

Figure 3. Zantiks Chamber side view

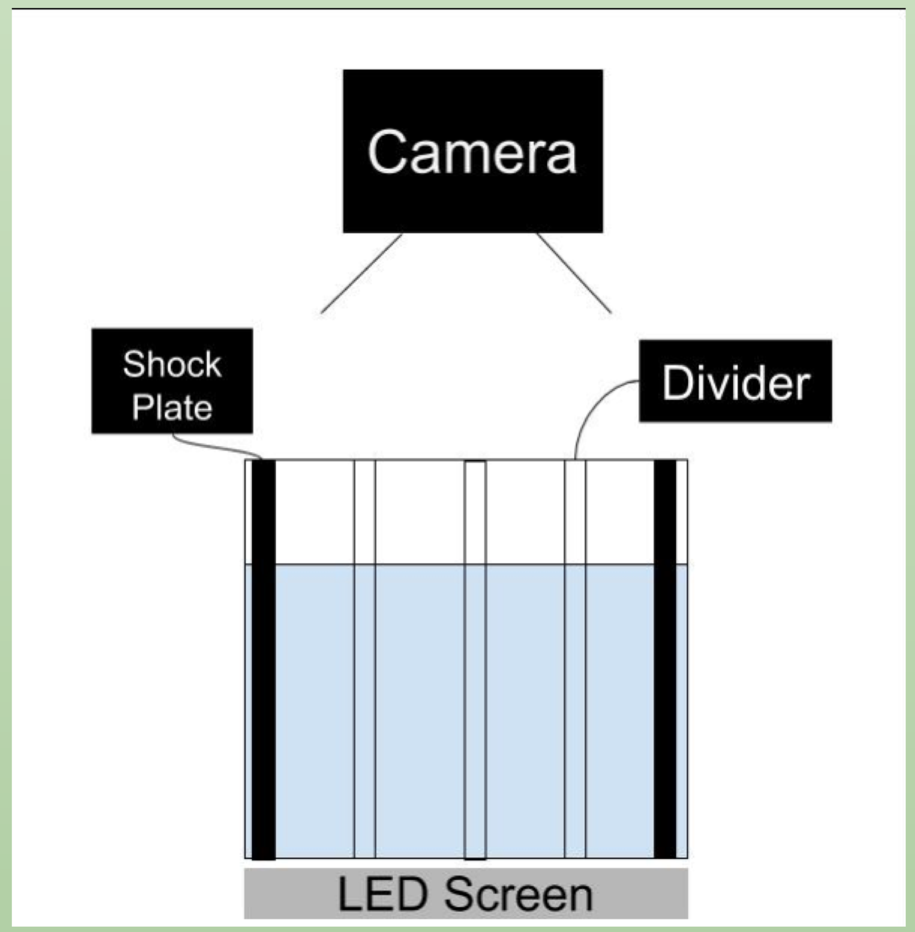
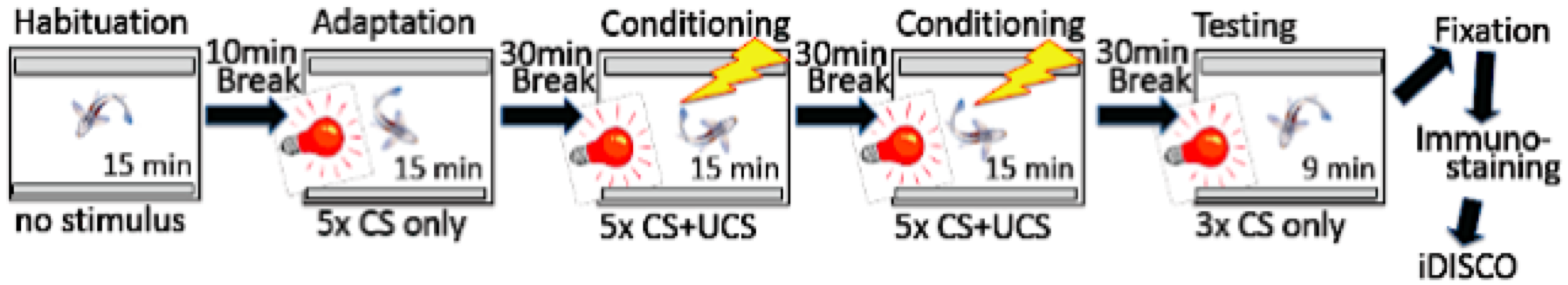


Figure 4. Light-Cued Paradigm



This figure shows where in our paradigm which stimulus will be used. CS stands for conditioned stimulus which is the LED screen light change. UCS is the Unconditioned Stimulus which is the electroshock. We hope that in the "Testing" phase, the fish will show an aversion to the light without needed the UCS.

Predictions

Completing this study will allow us to identify groups of calcium binding proteins expressing neurons that could be the causation of abnormal brain activity in the *shank3* mutant. This study will allow us to have a clearer background in various other studies. We predict that the *shank3* will show a different neurological reaction to the aversive stimulus compared to the wildtype Zebrafish.

Future Steps

In order to develop a more complete understanding of the zebrafish mutant model, we plan to carry out the fear conditioning paradigm. The overall goal is to get preliminary data to establish a clear and concise paradigm, while also allowing us to map neural activity. Another goal is to complete 3D imaging to present with our findings. These images can be very useful figures in the neuroscience field. We also aim to set up other paradigms. These could include social schooling, reward paradigm and spatial experiments. This current paradigm is vital as a foundation for future research.

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