Project Proposal

Project Title: The Role of RLX-33 on Social Behaviors of ARID1B Mutant Danio rerio

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Project Description:

Many different factors play a role in determining a person's behavior. Recent research has been uncovering the role of genetics and neurological processes in the development of one's personality and tendencies. The overall aim of this project is to identify if the antagonist of Relaxin-3, RLX-33, reverses the effects of the decreased social behavior exhibited by the fish with the ARID1B mutant that also expressed higher amounts of Relaxin-3. Furthermore, these findings will be connected to Autism Spectrum Disorder regarding potential treatment routes as lack of sociability is a common symptom of this disorder.

Background:

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder that affects the learning and social behaviors of millions of people around the world. Their delayed social maturation can make it difficult for them to be integrated members of society during their time in education, in their workplace, and in many other public settings. Unfortunately, there are no clear methods of treatment currently that can cure ASD, and many of the medications approved for this disorder primarily target one specific symptom, while causing many other negative drawbacks.

ARID1B

As research regarding the genetic basis of neurological disorders has been increasing, scientists have been using this information to improve early detection and treatment methods. Particularly, dozens of risk genes have been identified to significantly lead to more cases of ASD when a mutation occurs. However, research is ongoing about how the mutations exactly cause ASD symptoms.

One of the high-risk genes for ASD is the AT-rich interactive domain protein 1B (ARID1B) encoding gene. Previous studies have identified that mutations in this genome sequence often lead to haploinsufficiency of the protein. The primary function of the ARID1B protein is to regulate gene expression through chromatin remodeling, which refers to the adjustment of how tightly the DNA is wrapped, and hence, how much it is transcribed (ARID1B Gene, n.d.). This gene has been linked with many different intellectual disabilities, and one possible theory is that the ARID1B protein regulates neuron formation through chromatin remodeling in the brain (Sim et al., 2015).

In a previous study, an ASD risk gene was applied to Danio rerio through a protein truncation mutation of an ortholog gene of ARID1B in the fish (Capps et al., 2024). This resulted in abnormal amounts of activity, particularly during the day, as well as deficits in sociability amongst the other fish. These are both common indicators of ASD in Danio rerio behavior.

Relaxin-3

Amongst the Danio rerio that were given the ARID1B gene mutant, there was a significant increase in the amount of the Relaxin-3 and Urocortin-3 neuropeptides. Neuropeptides affect neuron activity and regulate the release of neurotransmitters. Relaxin-3 signaling in particular is associated with many different neurological processes relating to arousal, memory, mood, and anxiety. A previous study performed with mice identified that the mice that went through social interaction testing after being injected with the hormone Relaxin-3 performed significantly lower social approaches to other mice, as well as increased passive social contact. RLX-33 is an antagonist that can block this hormone from being expressed in the brain.

Experimental Design/Research Plan Goals:

The overall experiment can be broken up into two main objectives. The first goal is to create a line of Danio rerio fish with a mutant ARID1B gene. As the lab I am working in already has adult mutant ARID1B fish, the initial step would be to cross two of the fish from this clutch. To see if the mutation was successful in passing on to the offspring, I will run a PCR test for the ARID1B gene. The second goal is to inject the Danio rerio with the Relaxin-3 antagonist, RLX-33, and measure their social behavior compared to the mutant fish without the antagonist. So, the remaining fish with the ARID1B mutant will be injected with the antagonist around 5-7 days post fertilization, and then they will continue to be raised in the research institution's fish facilities until 21 days post fertilization, where they will undergo behavioral testing for sociability. They will be put into a behavior box set-up (Joo et al., 2021) and their sociability will be tested by recording how many times they initiate interactions with the other fish.

Risk/Safety Concerns:

There are no major risks or safety concerns with this project. All of the specific procedures that will be done throughout the experimentation process have been established beforehand in the lab I am working in, including the necessary precautions. The only potential safety concerns include working with the zebrafish and chemical substances. Still, any possible harm will be mitigated through the use of proper safety equipment (ex. gloves), constantly switching out materials that could cause contamination (ex. pipette tips), and also properly discarding used materials.

Data Analysis:

The data retrieved will include the success rates of the ARID1B mutation in the new zebrafish clutch, as well as the social preference index which is measured by the amount of interactions the fish initiate with their neighbor fish. The footage recorded using the behavior box will be processed through code in the LabVIEW software. A one-proportion z-test will be used to statistically analyze the success rate of the ARID1B mutations, and a two-sample t-test will be used to compare the difference in the means of social interactions in the control group versus the experimental group.

Timeline:

Phase 1: Cross ARID1B mutant zebrafish

Phase 2: Test if the offspring clutch successfully inherited the ARID1B mutant

Phase 3: Expose the fish to RLX-33

Phase 4: Run social behavior assays on zebrafish

References:

ARID1B gene: MedlinePlus Genetics. (n.d.). Retrieved January 10, 2024, from https://medlineplus.gov/genetics/gene/arid1b/

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