IRE1 Knockdown Leads to an Anxiety Phenotype in Drosophila and Changes in Protein Levels

Background – Anxiety and the Unfolded Protein Response

- Little is known about the molecular mechanisms behind anxiety.
- Anxiety is a failure to properly adapt to external stressors. In anxiety, as in other neuropsychiatric disorders, many proteins are dysregulated. The unfolded protein response (UPR) is responsible for regulating most proteins.
- The UPR leads to both protection and autophagy depending on severity of the stress.
- IRE1 is one of three branches of the UPR and is the most evolutionarily conserved pathway. Complete knockdown of IRE1 is not compatible with life.
- When IRE1 is activated, it leads to elevated levels of X-box binding protein 1 (XBP1) which then leads to changes in other proteins.
    - Two such proteins are Binding immunoglobulin Protein (BiP) and brain-derived neurotrophic factor (BDNF)
- BiP is a chaperone that works to refold proteins, so when IRE1 is activated, its levels increase. But if there are too many misfolded proteins, its levels decrease.
- BDNF plays an integral role in plasticity and is increased when IRE1 is activated.

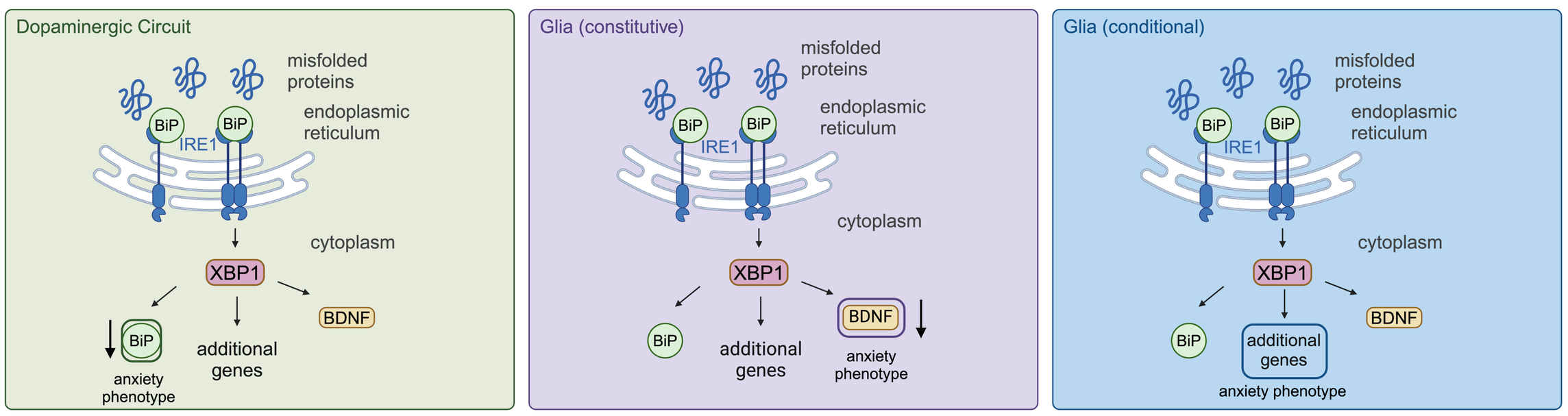

Figure 3. IRE1 knockdown in different locations and with different methods leads to varying impacts on proteins.

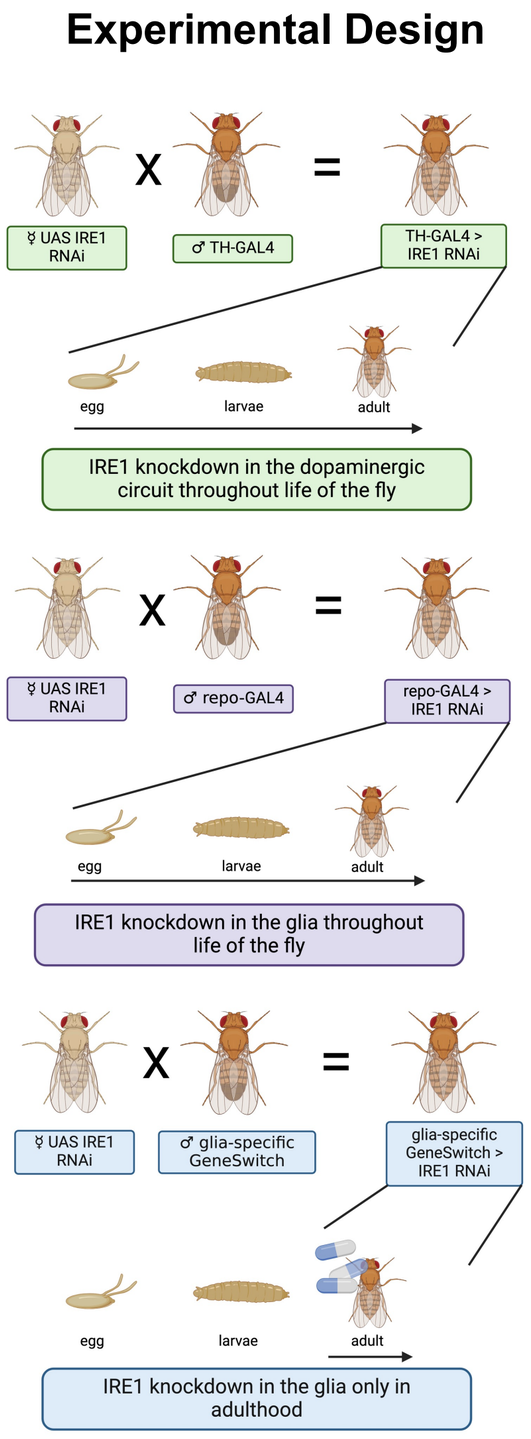

Figure 1. GAL4-UAS system crosses utilized in this experiment. This system was used so that IRE1 could be knocked down in a specific cell type

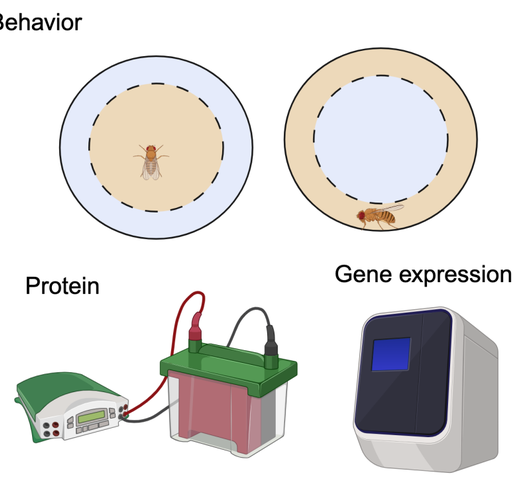

Figure 2. Experimental assays: Wall fallowing assay was used to determine behavioral phenotype, western blot was used to measure relative protein levels and qPCR was used to measure gene expression levels.

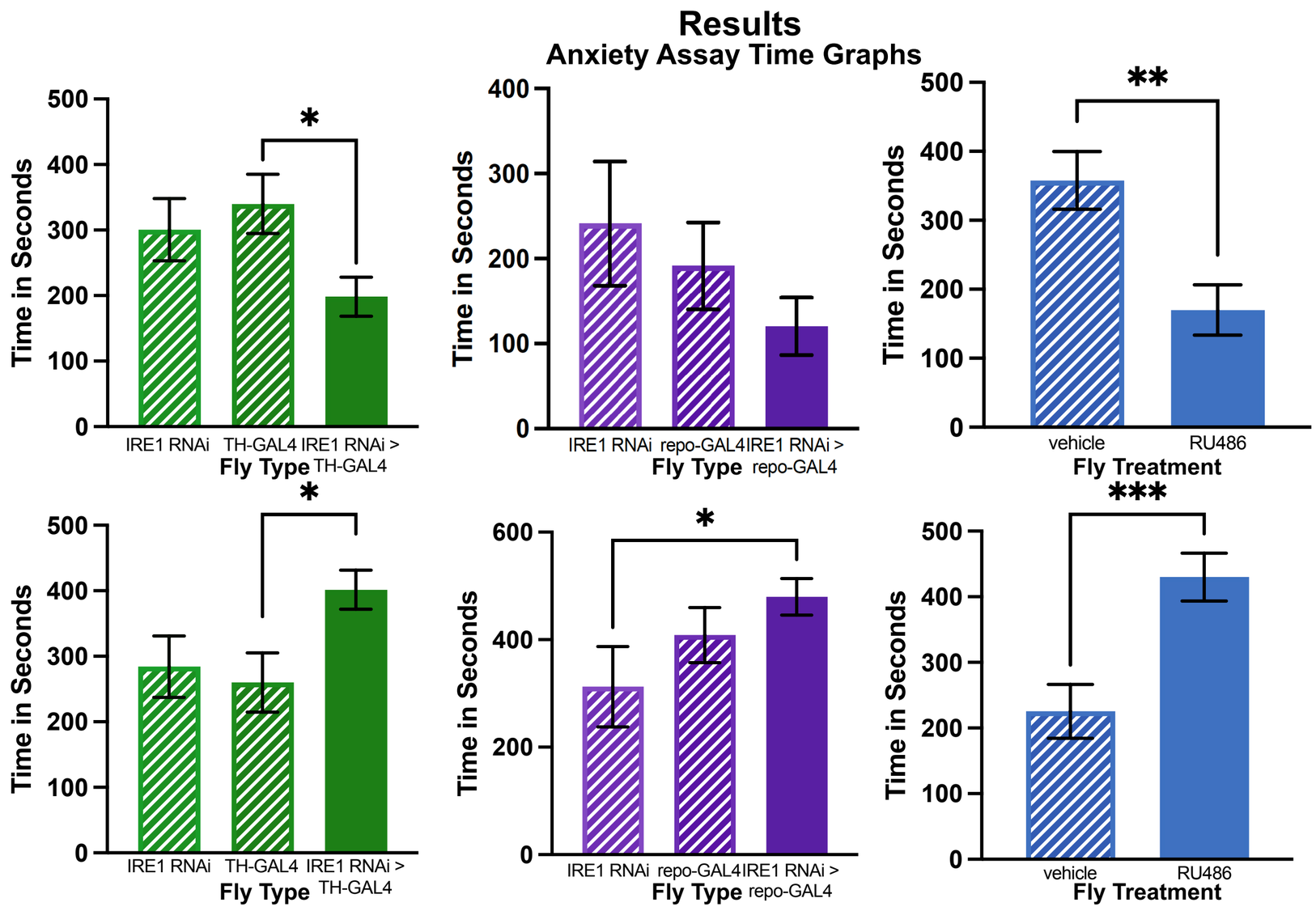

Figure 4. Anxiety assay time graphs show that all three fly crosses display anxiety phenotype, regardless of where or by which method IRE1 was knocked down.

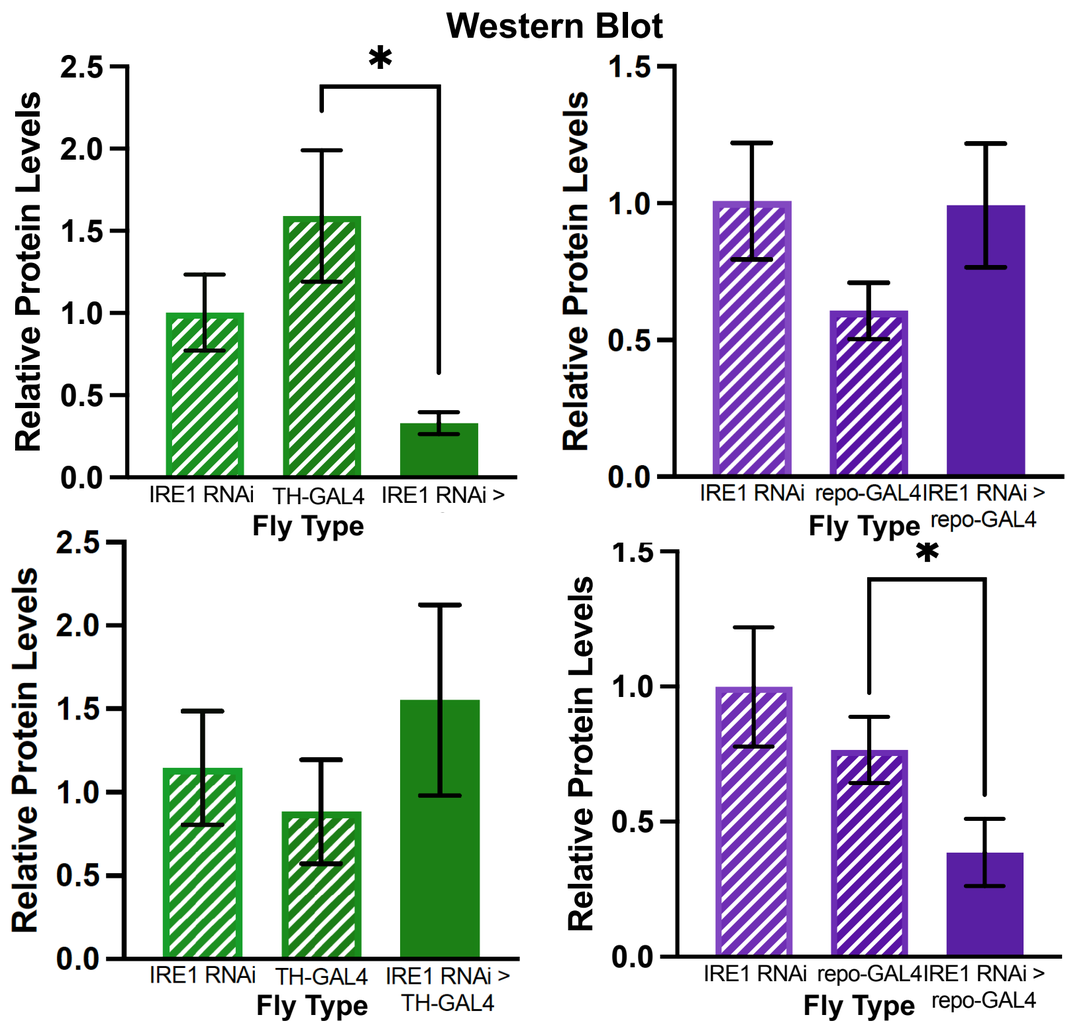

Figure 5. Western blots show that impacts on different protein varies depending on where IRE1 was knocked down.

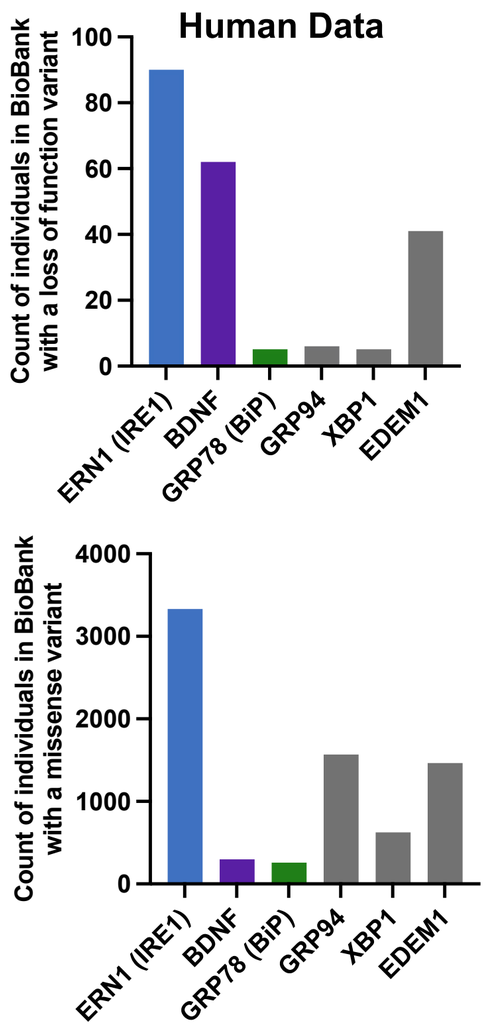

Figure 7. Data from the Penn Medicine BioBank suggests a human correlation based on genetic variants in the IRE1 pathway.

Discussion and Conclusions

- All three types of flies demonstrated a phenotype of anxiety.
- Changes in BiP and BDNF were dependent on where in the brain IRE1 was knocked down.
- BiP is directly responsible for refolding proteins. When IRE1 was knocked down in the dopaminergic circuit, there might have been too many misfolded proteins for BiP to handle so the cell would have died before BDNF could have been affected.
- BDNF regulates plasticity within glia which in turn relegates plasticity for the whole nervous system. This could be why knocking down IRE1 in the glia impacted BDNF rather than BiP.
- While more study is needed on the human data, many patients had loss of function or missense variants in ERN1 (IRE1 in humans). It is likely that these patients are predisposed to anxiety.

All schematics were created by the student using BioRender.