5-2023

Correlation Between Stress Coping Styles and Genetic Variation in the Adenosine Pathway of Zebrafish

Sydney Klucas

Correlation Between Stress Coping Styles and Genetic Variation in the Adenosine Pathway of Zebrafish

Sydney Klucas

Abstract—Anxiety is a common mental disorder and is caused in part by dysregulation of neurotransmitter systems. Specifically, the adenosine pathway has been correlated with anxiety disorders, but it is not yet known if genetic differences in this pathway are responsible for anxiety-related behavior. In this study, I used bioinformatic analyses (EdgeR and WGCNA) to investigate an RNA-sequencing dataset to assess differences in differential gene expression and gene co-expression networks within the adenosine pathway of zebrafish with different stress coping styles. These analyses included three brain regions: basolateral amygdala, hippocampus, and habenula. The differential gene expression analysis demonstrated that the adenosine deaminase gene was significantly proactive-biased while the adenosine receptor A2aa, acid phosphatase 1, and calcium binding protein 3 genes were significantly reactive-biased. WGCNA identified metabotropic glutamate receptors 5a and 5b genes as significantly correlated across multiple networks. The habenula brain region was linked to anxiety through the adenosine pathway by the differential gene expression analysis while the hippocampus was linked through weak preservation between the strains in WGCNA. The information gained from this project may be useful in uncovering the adenosine pathway's role in anxiety and be utilized to further investigate the role of adenosine receptor A2aa in anxiety behavior.

Index Terms—anxiety disorder, adenosine pathway, zebrafish, differential gene expression, EdgeR, weighted gene co-expression network analysis (WGCNA).

I. Introduction

Anxiety disorders are the most prevalent mental disorder and affect an estimated 284 million people globally (Dattani et al., 2021). Physiological effects of stress and anxiety, such as increased heart rate and breathing, sweaty palms, and shakiness, are caused in part by increased adrenaline and cortisol, as well as changes in neurotransmitter systems. Anxiety disorders can manifest from the dysregulation of these systems and disrupt day-to-day life (Martin et al., 2010). There are three main brain regions linked to anxiety, including the basolateral amygdala, hippocampus, and habenula. The basolateral amygdala is responsible in part for processing information related to fear, and hyperactivity within the region is associated with anxiety (Sharp, 2017). The hippocampus has an important role in learning and memory, and dysregulation of the region has been demonstrated to be correlated with anxiety disorders (Cominksi et al., 2014). The habenula is responsible in part for relaying information from the forebrain to the midbrain and hindbrain and regulates the expression of fear (Mathuru & Jesuthasan, 2013). These three brain regions may be useful to study in order to deepen the understanding of anxiety through the use of animal models.

Zebrafish are commonly used as an animal model to understand the pharmacological basis of anxiety because of their ease of genetic sequencing and anxiety behavior testing (Choi et al., 2021; Lachowicz et al., 2021; Stewart et al., 2012). Selective breeding has been utilized to investigate genetic and behavioral mechanisms of anxiety (Booher et al., 2012; Slattery et al., 2015). For example, the proactive and reactive strains of zebrafish differ in their stress and anxiety levels ranging from behavior to gene expression (Wong et al., 2012; Wong et al., 2015). Proactive zebrafish are characterized by displaying low anxiety-related behaviors while reactive zebrafish display more anxious behavior in several behavioral stress assays (Wong et al., 2012). Further, these two strains show distinct baseline neurotranscriptome profiles, including significant differences in transcription of several receptors and proteins in the adenosine pathway (Wong et al., 2015). Variation within the adenosine pathway has been linked to anxiety and may cause some people to be more susceptible to anxiety disorders than others (Alasmari, 2020; Calker et al., 2019).

Within the adenosine pathway, the adenosine molecule is an inhibitory neurotransmitter that binds to multiple adenosine receptors (A1, A2A, A2B, A3) and is degraded and produced by multiple enzymes. The binding of adenosine to the adenosine receptors causes a feeling of calm and is essential for the sleep-wake cycle and several other processes (Bjorness & Greene, 2009). Adenosine receptors A1 and A2A have been found to be implicated in anxiety in humans and various animal models (Childs et al., 2008; El Yacoubi et al., 2000; Jain et al., 1995; Maximino et al., 2011). Adenosine levels have been shown to increase in the brain after chronic stress, and adenosine levels are controlled by several enzymes (Zimmermann et al., 2016). Two of the main enzymes within the pathway include ecto-5'-nucleotidase, which produces adenosine by hydrolyzing adenosine monophosphate, and adenosine deaminase, which catalyzes the deamination of adenosine into inosine. Both ecto and cytosolic adenosine deaminase activity

have been found to decrease following a stressor, which may contribute to an increase in adenosine levels (Piato et al., 2011; Zimmermann et al., 2016). It is not yet known if the genetic differences in the adenosine pathway within the basolateral amygdala, hippocampus, and habenula brain regions are responsible in part for the reactive strain's increased anxiety and the proactive strain's decreased anxiety.

Transcriptome research such as RNA-sequencing can be used to study differences in gene expression associated with various diseases and disorders as well as study drug-induced changes in gene expression across the genome, which enables the identification of a drug target (Yang et al., 2020). RNA-sequencing techniques have been essential to investigate the genetic component of anxiety and other psychiatric disorders by identifying genes implicated in anxiety behavior (Su et al., 2021; Sun et al., 2021). In this study, I utilized RNA transcriptome data to conduct a bioinformatics-based study on the gene expression within the adenosine pathway in the basolateral amygdala, hippocampus, and habenula of proactive and reactive strains of zebrafish. I hypothesized that there will be significant differentially expressed genes and correlations in the adenosine pathway of different brain regions between the proactive and reactive strains of zebrafish.

II. Methods

A. Data Set Preparation

Prior to this study, an RNA-sequencing dataset was produced consisting of 11 proactive and 12 reactive zebrafish. For each zebrafish, there were transcriptome profiles established for three brain regions: dorsomedial telencephalon, dorsolateral telencephalon, and habenula. For simplicity, I will refer to the brain regions by the mammalian homologs: basolateral amygdala, hippocampus, and habenula, respectively (Parker et al., 2013). Reads were aligned to the zebrafish genome using GSNAP and quantified with HTSEQ (Anders et al., 2015; Wu & Nacu, 2010). From this dataset, I then generated an adenosine pathway specific dataset consisting of 42 genes using the follow gene ontology terms (GO IDs): G protein-coupled adenosine receptor signaling pathway (0001973), adenosine receptor binding (00316850), and adenosine biosynthesis process (0046086).

B. Bioinformatic Analyses

I conducted differential gene expression analyses using the EdgeR package in RStudio (Chen et al., 2016). The entire RNA-sequencing dataset was read into the program and then narrowed down to the list of adenosine pathway genes. I set strain, sex, and brain region as factors in a generalized linear model. I then combined all factors to create a design matrix to investigate the effect of strain and brain region on differential gene expression while controlling for sex. For the adenosine dataset, the program calculated the normalization and dispersion factors, and made contrasts between each group of strain and brain region. For each contrast, the program tested for significant differences in gene expression and calculated false discovery rates

(FDR) and counts per million (cpm) reads for all analyzed genes to be exported. I then created a multidimensional scaling (MDS) plot with strain and brain regions as factors to demonstrate gene expression clustering, and volcano plots for each brain region to demonstrate differential gene expression. The volcano plots have a significant FDR value of 0.05 and positive logFC value represents proactive-biased and negative logFC value represents reactive-biased gene expression.

I then investigated gene expression correlations using the weighted gene co-expression network analysis (WGCNA) package in RStudio and modified code from the following publication (Langfelder et al., 2011). I used WGCNA to characterize the adenosine signaling gene co-expression networks between the strains and brain regions. I first read the data files in cpm reads from the EdgeR analysis into the program and restricted to the Ensembl IDs from the gene ontology terms stated above. The program calculated network preservation statistics for each brain region in both strains and defined as follows: Preservation Z-summary scores greater than 10, between 10 and 2, and less than 2 are described as strongly, moderately, and weakly preserved, respectively. The program created circle plots by ordering the genes by weighted average connectivity and demonstrating positive correlation between genes in red and negative correlation in blue.

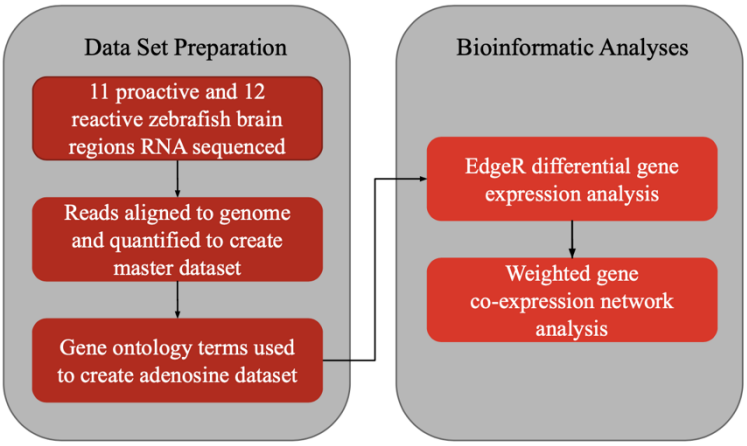

Figure 1. Bioinformatics workflow used to compare differential gene expression and gene co-expression networks within the basolateral amygdala, hippocampus, and habenula between proactive and reactive strains.

III. Results

A. EdgeR Differential Gene Expression Analysis

The EdgeR analysis produced an MDS plot that demonstrates the clustering of gene expression in the adenosine pathway of the different brain regions between the proactive and reactive strains (Figure 2). The proactive strain clustered lower than the reactive strain and gene expression within the habenula is clustered on the left while the basolateral amygdala and hippocampus are clustered on the right.

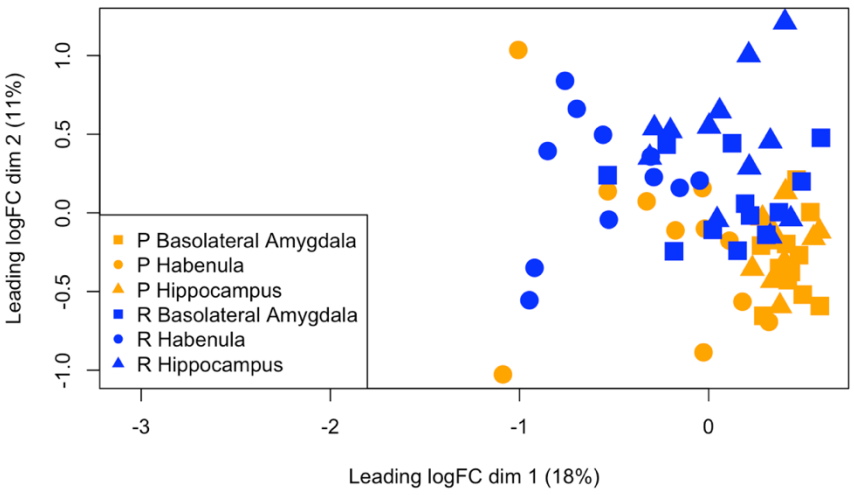

Figure 2. MDS plot of gene expression clustering within the adenosine pathway for each brain region between reactive (R) and proactive (P) strains. Color of the points represents the zebrafish strain (orange, proactive; blue, reactive) and shape of the points represents the brain region (square, basolateral amygdala; circle, habenula; triangle, hippocampus).

Within the basolateral amygdala and hippocampus brain regions, there were no significant differences in gene expression for any genes between the reactive and proactive fish. Within the habenula, the adenosine deaminase gene had significantly higher expression within proactive zebrafish ($p_{FDR} = 6.07x10^{-7}$), and the adenosine receptor A2aa gene had significantly higher expression within reactive zebrafish ($p_{FDR} = 0.0164$). Within all three regions, the adenosine deaminase gene had significantly higher expression within proactive zebrafish ($p_{FDR} = 1.50x10^{-8}$) while genes for acid phosphatase 1 and N-terminal EF-hand calcium binding protein 3 had significantly higher expression within reactive zebrafish ($p_{FDR} = 0.000251$ and $p_{FDR} = 0.00771$, respectively). Significant differentially expressed genes from EdgeR analysis are displayed within volcano plots in Figure 3 and in Table 1 with logFC and p_{FDR} values.

Brain Region	Gene Name	logFC	p_{FDR}
Habenula	Adenosine Deaminase	6.905	$6.07x10^{-7}$
	Adenosine Receptor A2aa	-3.092	0.0164
All Three Regions	Adenosine Deaminase	3.662	$1.50x10^{-8}$
	Acid Phosphatase 1	-0.4864	0.000251
	N-terminal EF-hand Calcium Binding Protein 3	-1.022	0.00771

Table 1. Significant differentially expressed genes ($p_{FDR} < 0.05$) between reactive and proactive strains. LogFC refers to the log fold change of gene expression with a positive value representing proactive-biased genes and a negative value representing reactive-biased genes.

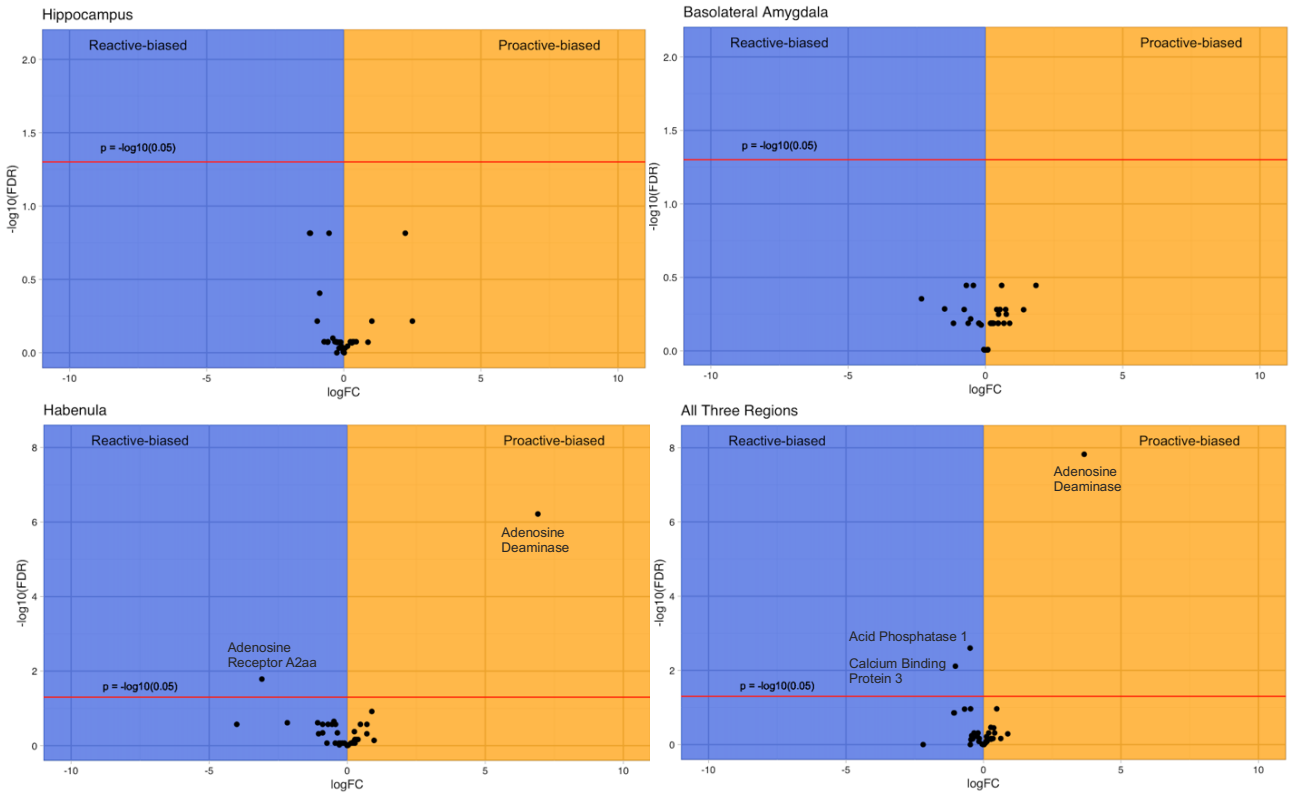

Figure 3. Volcano plots of differentially expressed genes for each brain region and all three regions between reactive and proactive strains. Significant differentially expressed genes FDR < 0.05. Blue left side represents reactive-biased genes, and orange right side represents proactive-biased genes.

B. Weighted Gene Co-Expression Network Analysis

WGCNA revealed that gene co-expression networks of the adenosine pathway are moderately preserved in the basolateral amygdala and habenula brain regions between reactive and proactive strains with preservation scores of 5.318 and 3.045, respectively. The gene co-expression networks within the hippocampus were weakly preserved between the strains with a preservation score of 0.3984 (Table 2). The largest quantity of significant gene expression correlations was within the hippocampus of both strains, followed by the basolateral amygdala, and the habenula with the least. Genes with significant correlations across multiple networks include *GRM* subtypes *GRM5A* and *GRM5B* that encode metabotropic glutamate receptors 5a and 5b, respectively (Figure 4).

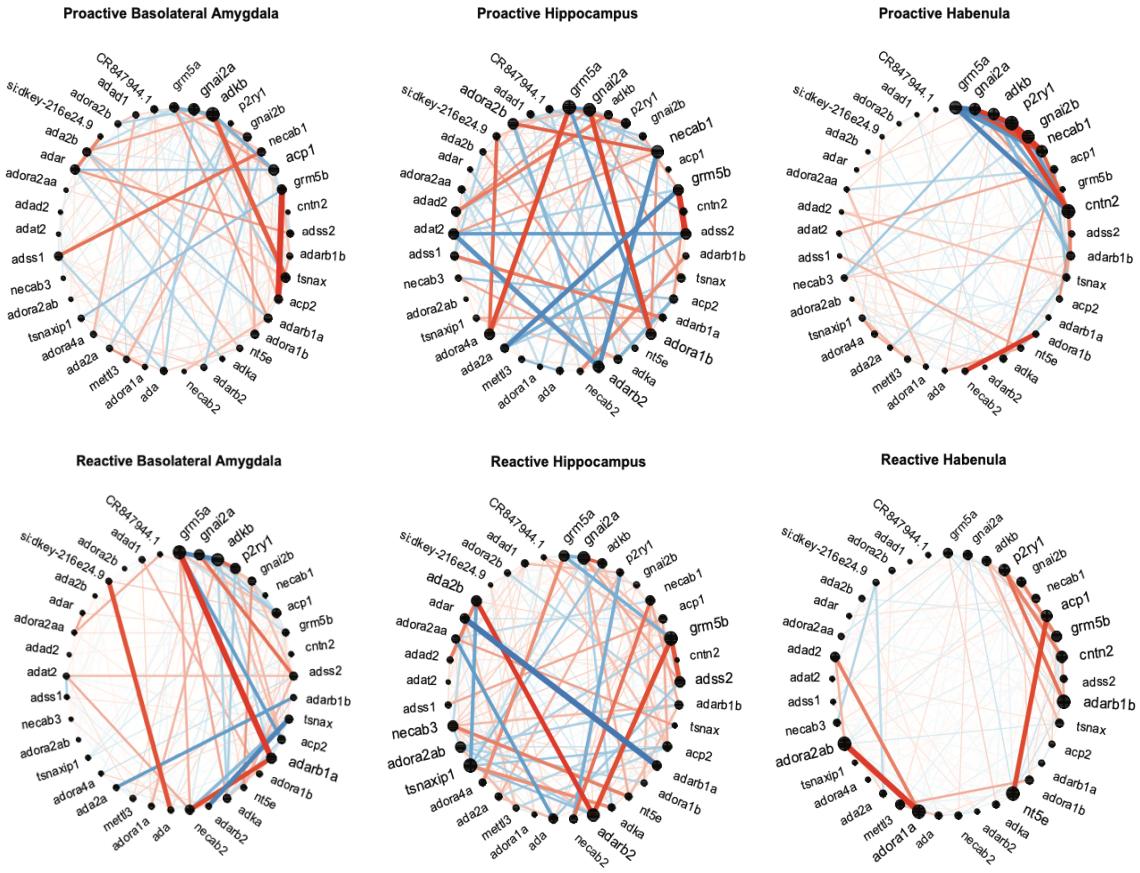

Figure 4. Adenosine pathway gene co-expression networks in each brain region of proactive and reactive strains. Direction of correlation (red = r > 0, blue = r < 0), correlation coefficient (thickness = | r |), and network centrality (diameter of black circle).

Brain Region	Preservation Score	P-Value
Basolateral Amygdala	5.318	6.097×10^{-8}
Hippocampus	0.3984	0.9402
Habenula	3.045	0.001597

Table 2. Preservation scores and significance of preservation ($p<0.05$) of adenosine pathway gene co-expression networks of a brain region between proactive and reactive strains.

IV. Discussion

This study has identified differences within the adenosine pathway between zebrafish strains and brain regions that may be correlated with anxiety behavior. The adenosine receptor A2aa is a central factor in many of the differentially expressed genes and correlations within gene networks, including increased expression of the N-terminal EF-hand calcium binding protein 3 gene and significantly correlated metabotropic glutamate receptor 5a and 5b genes. The adenosine deaminase gene showed increased expression within the proactive strain while the acid phosphatase 1 gene had increased expression within the reactive strain, indicating that the amount of adenosine in the three brain regions may also have a role in anxiety behavior. The habenula brain region was linked to anxiety behavior through the adenosine pathway by the differential gene expression analysis while the hippocampus was linked through weak preservation between the strains in WGCNA.

Adenosine receptors, specifically adenosine receptor A2A, has been shown to be a central factor in many of the differentially expressed genes and correlations within gene networks. Within the habenula, there was increased expression of the adenosine receptor A2aa in reactive zebrafish. This adenosine receptor is one of several receptors within the pathway that adenosine binds to. Increased expression may be correlated with the strain's increased anxiety behavior as overexpression of this adenosine receptor has been linked to anxiety and depression in rats, and adenosine receptor A2A agonists have demonstrated potential for treatment of anxiety disorders (Coelho et al., 2014; Guerrero, 2018). The analyses also demonstrated an increased expression of the N-terminal EF-hand calcium binding protein 3 gene within the reactive zebrafish. This calcium binding protein has been shown to modulate the function of the adenosine receptor A2A through cell surface expression (Canela et al., 2007). This modulation may impact adenosine binding to the adenosine receptor A2A and cause a downward effect in reactive zebrafish that may increase anxiety behavior. The combined effects of the overexpression of adenosine receptor A2aa and the calcium binding protein within the reactive strain may be significant in its high levels of anxiety behavior compared to the proactive strain. This calcium binding protein has also been shown to modulate the function of other receptors involved in the adenosine pathway, including metabotropic glutamate (mGlu) receptors 5a and 5b (Canela et al., 2009).

Genes with significant correlations across multiple networks in the WGCNA analysis included mGlu receptors 5a and 5b. In addition to the mGlu receptors' interactions with the calcium binding protein, they have been shown to be involved in crosstalk with adenosine receptor A2A through multiple mechanisms, including synaptic transmission, neurotransmitter release, and behavioral effects (Ferré et al., 2002; Kachroo et al., 2005; León-Navarro et al., 2019). These findings indicates that the calcium binding protein and mGlu receptors 5a and 5b may have downstream effects on the adenosine receptor A2A that are significant for differences in anxiety behavior between the reactive and proactive strains.

In addition to modulation of adenosine receptor A2A, differences in adenosine levels may cause altered anxiety behavior between the strains as the binding of adenosine to adenosine

receptors causes a calming effect (Bjorness & Greene, 2009). In the differential gene expression analysis, proactive zebrafish had increased expression of adenosine deaminase within the habenula as well as within combined analyses of all three brain regions. This may indicate that there are lower levels of adenosine within the three brain regions of the proactive strain since the adenosine deaminase enzyme irreversibly catalyzes the deamination of adenosine into inosine. On the other hand, adenosine levels in the reactive strain may be high because this strain was shown to have increased expression of the acid phosphatase 1 gene. The acid phosphatase enzyme converts adenosine monophosphate into adenosine by catalyzing the hydrolysis reaction under acidic conditions, and the overexpression of this enzyme may increase adenosine levels within the brain of the reactive strain. The overexpression of adenosine deaminase and acid phosphatase both have roles within the immune response but have not yet been experimentally implicated in anxiety (Halaby et al., 2001; Qu et al., 2022; Tamura et al., 2016). Although the metabolism of adenosine has not been linked to anxiety directly, chronic stress has been shown to significantly increase adenosine levels in the brains of zebrafish and was theorized to be a compensatory mechanisms to counteract high levels of stress (Zimmermann et al., 2016). With this reasoning, high levels of adenosine in reactive zebrafish may not cause anxiety behavior but instead be a mechanism to decrease anxiety. Higher concentrations of adenosine would allow for more adenosine receptor interactions and produce a calming effect. Low levels of adenosine may be present within the proactive strain since they have a low anxiety baseline and do not require a mechanism to decrease anxiety.

The differences in adenosine pathway activity between strains including adenosine receptor A2aa expression and predicted adenosine levels are occurring in certain brain regions. Of the three brain regions tested in the differential gene expression analysis, the habenula was shown to have significant differences in gene expression between the strains. These differences included increased expression of the adenosine receptor A2aa in reactive zebrafish and increased expression of adenosine deaminase in proactive zebrafish. This may suggest that the habenula has a key role in anxiety through the adenosine pathway with similar results linking adenosine receptor A2A to depressive behaviors within the habenula (Wang et al., 2023). On the other hand, WGCNA demonstrated that gene co-expression networks within the adenosine pathway was weakly preserved in the hippocampus between proactive and reactive strains. The difference in networks between the strains may indicate that the hippocampus also plays a role in anxiety through the adenosine pathway. This has been supported with research correlating anxiety with the inhibition of adenosine receptors A2A within the ventral hippocampus (Xu et al., 2022). The information gained from this project may be useful in uncovering the adenosine pathway's role in anxiety and be utilized to further investigate the role of adenosine receptor A2aa in anxiety behavior through further research.

Acknowledgements

I would like to thank Dr. Ryan Wong and Dr. Levi Storks for training and mentoring me throughout this project as well as the Wong lab for helpful insight and discussion. This study was supported by funds from the National Institutes of Health (R15MH113074) to Dr. Ryan Wong.

References

Alasmari, F. (2020). Caffeine induces neurobehavioral effects through modulating neurotransmitters. *Saudi Pharmaceutical Journal, 28*(4), 445-451. doi:10.1016/j.jsps.2020.02.005

Anders, S., Pyl, P. T., & Huber, W. (2015). HTSeq—A Python framework to work with high-throughput sequencing data. *Bioinformatics*, *31*(2), 166–169. https://doi.org/10.1093/bioinformatics/btu638

Bjorness, T. E., & Greene, R. W. (2009). Adenosine and sleep. *Current Neuropharmacology*, *7*(3), 238–245. https://doi.org/10.2174/157015909789152182

Booher, W. C., Hall, L. A., Thomas, A. L., Merhroff, E. A., Reyes Martínez, G. J., Scanlon, K. E., Lowry, C. A., & Ehringer, M. A. (2021). Anxiety-related defensive behavioral responses in mice selectively bred for high and low activity. *Genes, Brain and Behavior*, *20*(7). https://doi.org/10.1111/gbb.12730

Calker, D., Biber, K., Domschke, K., & Serchov, T. (2019). The role of adenosine receptors in mood and anxiety disorders. *Journal of Neurochemistry, 151*(1), 11-27. doi:10.1111/jnc.14841

Canela, L., Fernández-Dueñas, V., Albergaria, C., Watanabe, M., Lluís, C., Mallol, J., Canela, E. I., Franco, R., Luján, R., & Ciruela, F. (2009). The association of metabotropic glutamate receptor type 5 with the neuronal Ca2+-binding protein 2 modulates receptor function. *Journal of Neurochemistry*, *111*(2), 555–567. https://doi.org/10.1111/j.1471-4159.2009.06348.x

Canela, L., Luján, R., Lluís, C., Burgueño, J., Mallol, J., Canela, E. I., Franco, R., & Ciruela, F. (2007). The neuronal Ca2+-binding protein 2 (Necab2) interacts with the adenosine A2A receptor and modulates the cell surface expression and function of the receptor. *Molecular and Cellular Neuroscience*, *36*(1), 1–12. https://doi.org/10.1016/j.mcn.2007.05.007

Chen Y, Lun AAT, Smyth GK (2016). "From reads to genes to pathways: differential expression analysis of RNA-Seq experiments using Rsubread and the edgeR quasi-likelihood pipeline." *F1000Research*, **5**, 1438. doi:10.12688/f1000research.8987.2.

Choi, T.-Y., Choi, T.-I., Lee, Y.-R., Choe, S.-K., & Kim, C.-H. (2021). Zebrafish as an animal model for biomedical research. *Experimental & Molecular Medicine*, *53*(3), 310–317. https://doi.org/10.1038/s12276-021-00571-5

Coelho, J. E., Alves, P., Canas, P. M., Valadas, J. S., Shmidt, T., Batalha, V. L., Ferreira, D. G., Ribeiro, J. A., Bader, M., Cunha, R. A., Do Couto, F. S., & Lopes, L. V. (2014). Overexpression of adenosine a2a receptors in rats: Effects on depression, locomotion, and anxiety. *Frontiers in Psychiatry*, *5*. https://doi.org/10.3389/fpsyt.2014.00067

Cominski, T. P., Jiao, X., Catuzzi, J. E., Stewart, A. L., & Pang, K. C. H. (2014). The role of the hippocampus in avoidance learning and anxiety vulnerability. *Frontiers in Behavioral Neuroscience*, *8*. https://www.frontiersin.org/articles/10.3389/fnbeh.2014.00273

Dattani, S., Ritchie, H., & Roser, M. (2021). Mental health. *Our World in Data*. https://ourworldindata.org/mental-health

El Yacoubi, M., Ledent, C., Parmentier, M., Costentin, J., & Vaugeois, J.-M. (1999). Selective adenosine A2A receptor antagonists are potential antidepressants: Evidence based on the activity of SCH 58261 and A2A knockout mice in screening procedures. *European Neuropsychopharmacology*, *9*, 249. https://doi.org/10.1016/S0924-977X(99)80232-6

Ferré, S., Karcz-Kubicha, M., Hope, B. T., Popoli, P., Burgueño, J., Gutiérrez, M. A., Casadó, V., Fuxe, K., Goldberg, S. R., Lluis, C., Franco, R., & Ciruela, F. (2002). Synergistic interaction between adenosine A2A and glutamate mGlu5 receptors: Implications for striatal neuronal function. *Proceedings of the National Academy of Sciences*, *99*(18), 11940–11945. https://doi.org/10.1073/pnas.172393799

Flinn, A. M., & Gennery, A. R. (2018). Adenosine deaminase deficiency: A review. *Orphanet Journal of Rare Diseases*, *13*(1), 65. https://doi.org/10.1186/s13023-018-0807-5

George, C. X., Gan, Z., Liu, Y., & Samuel, C. E. (2011). Adenosine deaminases acting on rna, rna editing, and interferon action. *Journal of Interferon & Cytokine Research*, *31*(1), 99–117. https://doi.org/10.1089/jir.2010.0097

Guerrero, A. (2018). A2a adenosine receptor agonists and their potential therapeutic applications. An update. *Current Medicinal Chemistry*, *25*(30), 3597–3612. https://doi.org/10.2174/0929867325666180313110254

Halaby, R., Abdollahi, J., & Martinez, M. L. (2001). Acid phosphatase activity in human breast tumors. *Breast Cancer Research*, *3*(2), E002. https://doi.org/10.1186/bcr296

Jain, N., Kemp, N., Adeyemo, O., Buchanan, P., & Stone, T. W. (1995). Anxiolytic activity of adenosine receptor activation in mice. *British Journal of Pharmacology*, *116*(3), 2127–2133. https://doi.org/10.1111/j.1476-5381.1995.tb16421.x

Kachroo, A., Orlando, L. R., Grandy, D. K., Chen, J.-F., Young, A. B., & Schwarzschild, M. A. (2005). Interactions between metabotropic glutamate 5 and adenosine a2a receptors in normal and parkinsonian mice. *Journal of Neuroscience*, *25*(45), 10414–10419. https://doi.org/10.1523/JNEUROSCI.3660-05.2005

Lachowicz, J., Niedziałek, K., Rostkowska, E., Szopa, A., Świąder, K., Szponar, J., & Serefko, A. (2021). Zebrafish as an animal model for testing agents with antidepressant potential. *Life*, *11*(8), 792. https://doi.org/10.3390/life11080792

Langfelder, P., Luo, R., Oldham, M. C., & Horvath, S. (2011). Is my network module preserved and reproducible? *PLoS Computational Biology*, *7*(1), e1001057. https://doi.org/10.1371/journal.pcbi.1001057

León-Navarro, D. A., Albasanz, J. L., & Martín, M. (2019). Functional cross-talk between adenosine and metabotropic glutamate receptors. *Current Neuropharmacology*, *17*(5), 422–437. https://doi.org/10.2174/1570159X16666180416093717

Martin, E. I., Ressler, K. J., Binder, E., & Nemeroff, C. B. (2010). The neurobiology of anxiety disorders: Brain imaging, genetics, and psychoneuroendocrinology. *Clinics in Laboratory Medicine, 30*(4), 865-891. doi:10.1016/j.cll.2010.07.006

Mathuru, A. S., & Jesuthasan, S. (2013). The medial habenula as a regulator of anxiety in adult zebrafish. *Frontiers in Neural Circuits*, *7*, 99. https://doi.org/10.3389/fncir.2013.00099

Maximino, C., Lima, M. G., Olivera, K. R. M., Picanço-Diniz, D. L. W., & Herculano, A. M. (2011). Adenosine a1, but not a2, receptor blockade increases anxiety and arousal in zebrafish: Adenosine, anxiety and arousal. *Basic & Clinical Pharmacology & Toxicology*, *109*(3), 203–207. https://doi.org/10.1111/j.1742-7843.2011.00710.x

Parker, M. O., Brock, A. J., Walton, R. T., & Brennan, C. H. (2013). The role of zebrafish (Danio rerio) in dissecting the genetics and neural circuits of executive function. *Frontiers in Neural Circuits*, *7*, 63. https://doi.org/10.3389/fncir.2013.00063

Piato, A. L., Rosemberg, D. B., Capiotti, K. M., Siebel, A. M., Herrmann, A. P., Ghisleni, G., Vianna, M. R., Bogo, M. R., Lara, D. R., & Bonan, C. D. (2011). Acute restraint stress in zebrafish: Behavioral parameters and purinergic signaling. *Neurochemical Research*, *36*(10), 1876–1886. https://doi.org/10.1007/s11064-011-0509-z

Qu, Y., Dunn, Z. S., Chen, X., MacMullan, M., Cinay, G., Wang, H., Liu, J., Hu, F., & Wang, P. (2022). Adenosine deaminase 1 overexpression enhances the antitumor efficacy of chimeric antigen receptor-engineered t cells. *Human Gene Therapy*, *33*(5–6), 223–236. https://doi.org/10.1089/hum.2021.050

Riquier, A. J., & Sollars, S. I. (2017). Microglia density decreases in the rat rostral nucleus of the solitary tract across development and increases in an age-dependent manner following denervation. *Neuroscience*, *355*, 36–48. https://doi.org/10.1016/j.neuroscience.2017.04.037

Rodrigues, R. J., Alfaro, T. M., Rebola, N., Oliveira, C. R., & Cunha, R. A. (2005). Co-localization and functional interaction between adenosine A2A and metabotropic group 5 receptors in glutamatergic nerve terminals of the rat striatum. *Journal of Neurochemistry*, *92*(3), 433–441. https://doi.org/10.1111/j.1471-4159.2004.02887.x

Sharp, B. M. (2017). Basolateral amygdala and stress-induced hyperexcitability affect motivated behaviors and addiction. *Translational Psychiatry*, *7*(8), e1194–e1194. https://doi.org/10.1038/tp.2017.161

Slattery, D. A., Naik, R. R., Grund, T., Yen, Y.-C., Sartori, S. B., Füchsl, A., Finger, B. C., Elfving, B., Nordemann, U., Guerrini, R., Calo, G., Wegener, G., Mathé, A. A., Singewald, N., Czibere, L., Landgraf, R., & Neumann, I. D. (2015). Selective breeding

for high anxiety introduces a synonymous snp that increases neuropeptide s receptor activity. *Journal of Neuroscience*, *35*(11), 4599–4613. https://doi.org/10.1523/JNEUROSCI.4764-13.2015

Song, B., Shiromoto, Y., Minakuchi, M., & Nishikura, K. (2022). The role of RNA editing enzyme ADAR1 in human disease. *WIREs RNA*, *13*(1). https://doi.org/10.1002/wrna.1665

Stewart, A., Gaikwad, S., Kyzar, E., Green, J., Roth, A., & Kalueff, A. V. (2012). Modeling anxiety using adult zebrafish: A conceptual review. *Neuropharmacology*, *62*(1), 135–143. https://doi.org/10.1016/j.neuropharm.2011.07.037

Su, X., Li, W., Lv, L., Li, X., Yang, J., Luo, X.-J., & Liu, J. (2021). Transcriptome-wide association study provides insights into the genetic component of gene expression in anxiety. *Frontiers in Genetics*, *12*, 740134. https://doi.org/10.3389/fgene.2021.740134

Sun, Y., Ma, L., Chen, J., Wang, W., Peng, S., Cheng, Y., Zhang, Y., Chen, J., & Ju, P. (2021). RNA-seq co-expression network analysis reveals anxiolytic behavior of mice with Efnb2 knockout in parvalbumin+ neurons. *Molecular Brain*, *14*(1), 118. https://doi.org/10.1186/s13041-021-00829-z

Tamura, R., Ohta, H., Satoh, Y., Nonoyama, S., Nishida, Y., & Nibuya, M. (2016). Neuroprotective effects of adenosine deaminase in the striatum. *Journal of Cerebral Blood Flow & Metabolism*, *36*(4), 709–720. https://doi.org/10.1177/0271678X15625077

Tartar, J. L., Hiffernan, F. S., Freitas, K. E., Fins, A. I., & Banks, J. B. (n.d.). A functional adenosine deaminase polymorphism associates with evening melatonin levels and sleep quality. *Journal of Circadian Rhythms*, *19*, 5. https://doi.org/10.5334/jcr.209

Wang, M., Li, P., Li, Z., Da Silva, B. S., Zheng, W., Xiang, Z., He, Y., Xu, T., Cordeiro, C., Deng, L., Dai, Y., Ye, M., Lin, Z., Zhou, J., Zhou, X., Ye, F., Cunha, R. A., Chen, J., & Guo, W. (2023). Lateral septum adenosine A2A receptors control stress-induced depressive-like behaviors via signaling to the hypothalamus and habenula. *Nature Communications*, *14*(1), 1880. https://doi.org/10.1038/s41467-023-37601-x

Wong, R. Y., Lamm, M. S., & Godwin, J. (2015). Characterizing the neurotranscriptomic states in alternative stress coping styles. *BMC Genomics, 16*(1). doi:10.1186/s12864-015-1626-x

Wong, R. Y., Perrin, F., Oxendine, S. Y., Kezios, Z. U., Sawyer, S. D., Zhou, L. U., Goodwin, J. (2012). Comparing behavioral responses across multiple assays of stress and anxiety in zebrafish (Danio rerio). *Behaviour, 149*(10-12), 1205-1240. doi:10.1163/1568539x-00003018

Wu, T. D., & Nacu, S. (2010). Fast and SNP-tolerant detection of complex variants and splicing in short reads. *Bioinformatics*, *26*(7), 873–881. https://doi.org/10.1093/bioinformatics/btq057

Xu, Y., Ning, Y., Zhao, Y., Peng, Y., Luo, F., Zhou, Y., & Li, P. (2022). Caffeine functions by inhibiting dorsal and ventral hippocampal adenosine 2a receptors to modulate memory

and anxiety, respectively. *Frontiers in Pharmacology*, *13*, 807330. https://doi.org/10.3389/fphar.2022.807330

Yang, X., Kui, L., Tang, M., Li, D., Wei, K., Chen, W., Miao, J., & Dong, Y. (2020). High-throughput transcriptome profiling in drug and biomarker discovery. *Frontiers in Genetics*, *11*. https://www.frontiersin.org/articles/10.3389/fgene.2020.00019

Zimmermann, F. F., Altenhofen, S., Kist, L. W., Leite, C. E., Bogo, M. R., Cognato, G. P., & Bonan, C. D. (2016). Unpredictable chronic stress alters adenosine metabolism in zebrafish brain. *Molecular Neurobiology*, *53*(4), 2518–2528. https://doi.org/10.1007/s12035-015-9270-7