

Unraveling Sexual Dimorphism in Rat Brain Gene Expression during Early Developmental Stages

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Introduction

Sex differences are a prominent feature of developmental brain diseases [1]. Understanding when these differences occur could shed light on the mechanisms of neurodevelopmental disorders. Furthermore, females are often excluded or overlooked in gene expression analyses. Therefore, we sought to investigate whether there are differences in gene expression between male and female rat brain development. To do this, we used RNA-seq data from Yu et al. (2014) [2]. From these data, we focused on juvenile and adolescent rats to capture early development. We hypothesized that visible sex differences in gene expression would emerge in late development, as that is when sex differences become phenotypically apparent.

Methods

We used the RNA-sequencing dataset from Yu et al. (2014), which includes 11 organ tissues at four life stages in rats, focusing here on brain tissue at the first two stages. The dataset was normalized using the TMM (Trimmed Mean of M-values) method in edgeR. Genes with fewer than two samples showing counts greater than 1 CPM were excluded. The remaining read counts were transformed into $\log_2$ values for easier interpretation. To assess sex differences in gene expression, we generated two volcano plots comparing male and female rats at 2 and 6 weeks of age, and summarized the number of significant genes in a bar plot. To further explore the data, two PCA plots were generated to visualize differences between male and female subgroups at 2 and 6 weeks, and a heatmap to show expression patterns of sex-biased genes in the brain across these two stages.

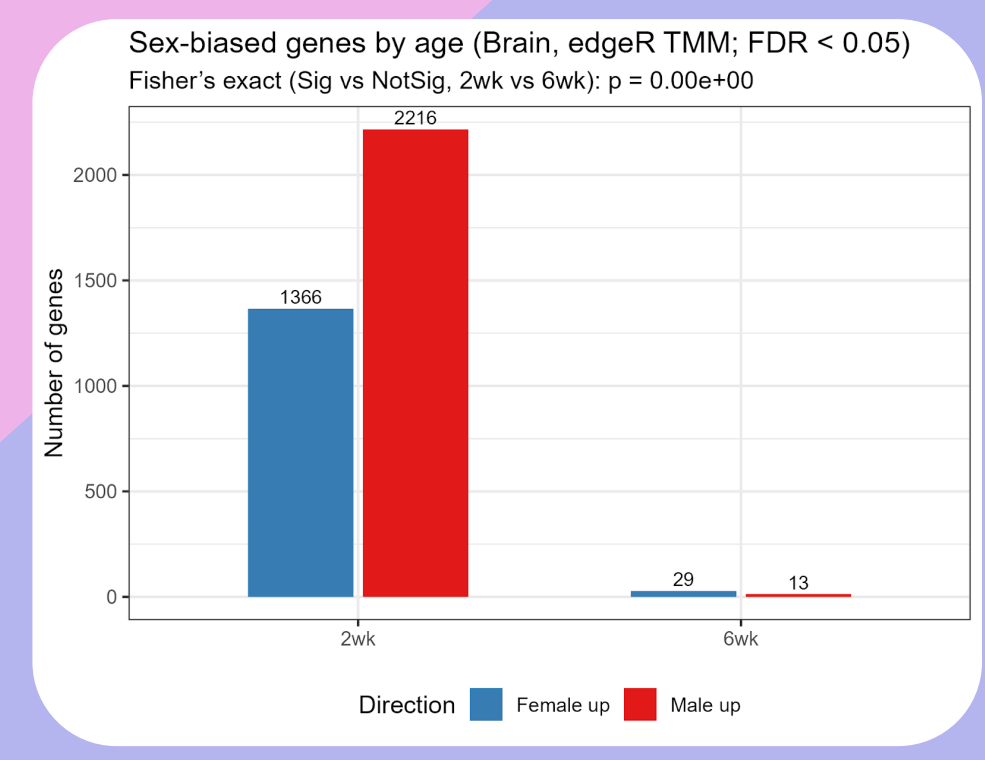
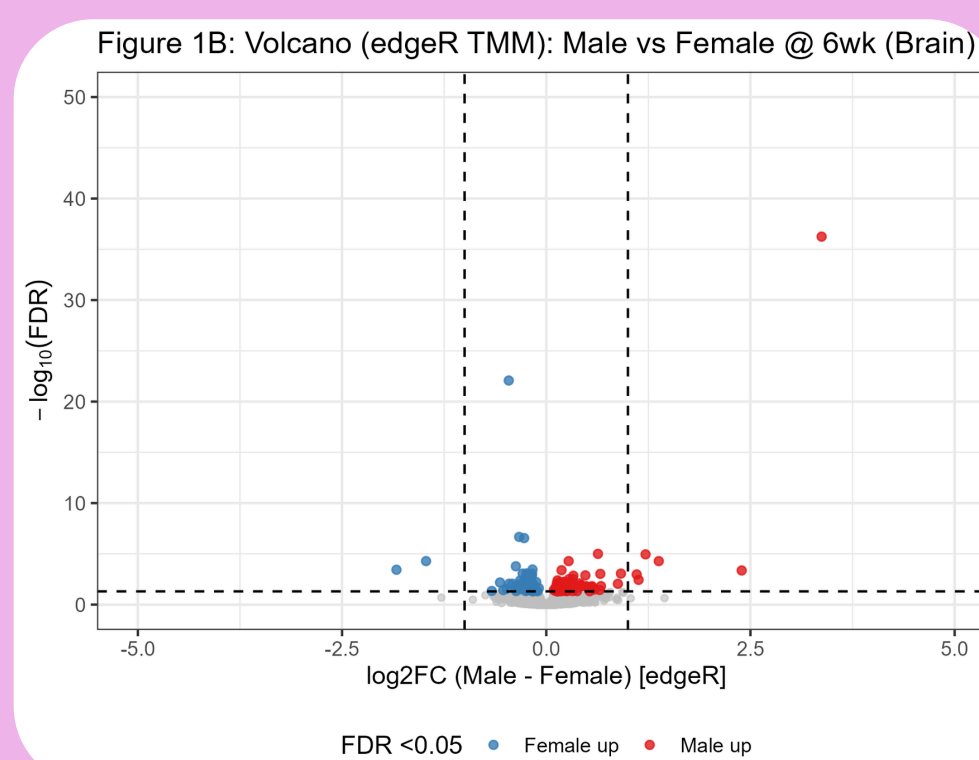
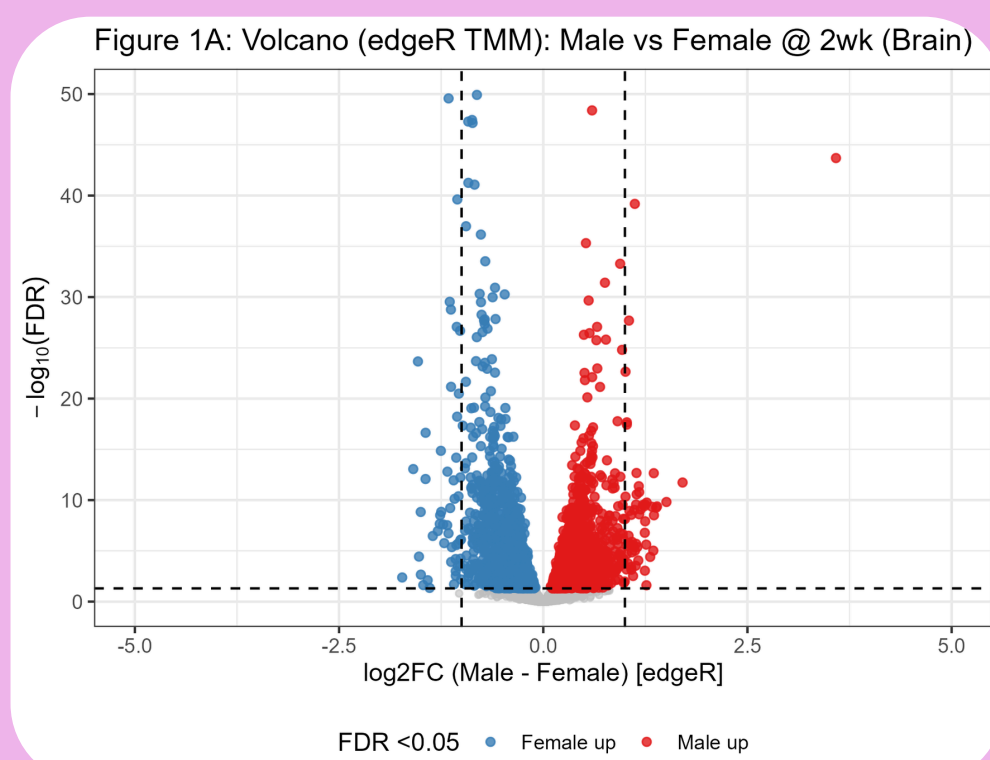


Figure 1. Volcano plots of sex-biased gene expression in rat brain at week 2 (1A) and week 6 (1B). Each dot represents a gene. The x-axis shows the $\log_2$ fold-change (Male - Female), and the y-axis shows the $-\log_{10}$ FDR (false discovery rate-adjusted p-value) from differential expression testing. Genes with FDR < 0.05 are highlighted: red = male-biased, blue = female-biased, grey = not significant. Dashed lines indicate common cutoffs (vertical: $\pm 1 \log_2$ FC; horizontal: FDR = 0.05).

Figure 2. Numbers of sex-biased genes in rat brain at 2 and 6 weeks. Bars show counts of significantly sex-biased genes (FDR < 0.05, edgeR TMM). Red = male-biased, blue = female-biased. At 2 weeks, thousands of genes show sex-biased expression, whereas by 6 weeks only a few dozen remain. The difference in proportions of significant genes is highly significant (Fisher's exact test, $p < 1 \times 10^{-16}$).

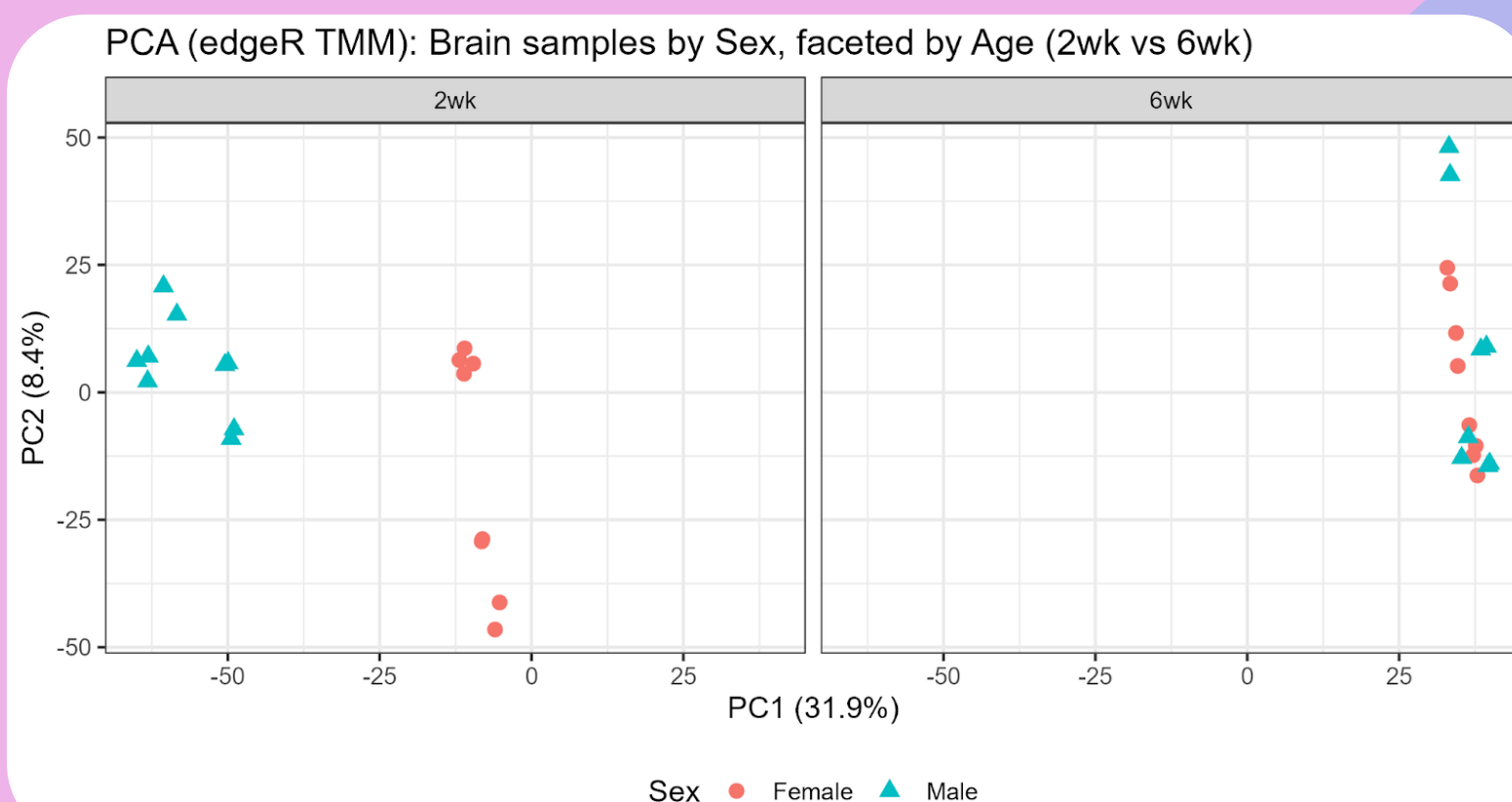


Figure 3. PCA of brain samples filtered by sex at 2 and 6 weeks. Each point represents one sample, positioned according to the first two principal components of variance in gene expression (edgeR TMM logCPM). Colors and shapes indicate sex. Panels are faceted by age group. At 2 weeks, males and females show partial separation, while by 6 weeks the separation is less pronounced, consistent with the reduction in sex-biased genes observed in differential expression analyses.

Discussion

There is a greater sex difference in brain tissue gene expression in 2-week-old rats than in 6-week-old rats. This finding was unexpected, as it contrasts with our original hypothesis. One possible explanation is the surge of testosterone in newborn male rats [3], which directly alters gene expression during early postnatal development. Although male and female brains eventually diverge phenotypically, the transcriptional differences appear to diminish once testosterone levels return to baseline, when female-typical programs are no longer suppressed [4]. These results highlight the importance of considering developmental stage in the design of drugs targeting the brain. Future studies could examine how fluctuating testosterone levels influence gene expression in the developing brain. Furthermore, studies may focus on gene ontology and translate this into human orthologs, furthering understanding of human brain development.



References, R-Code and Supplementary data :

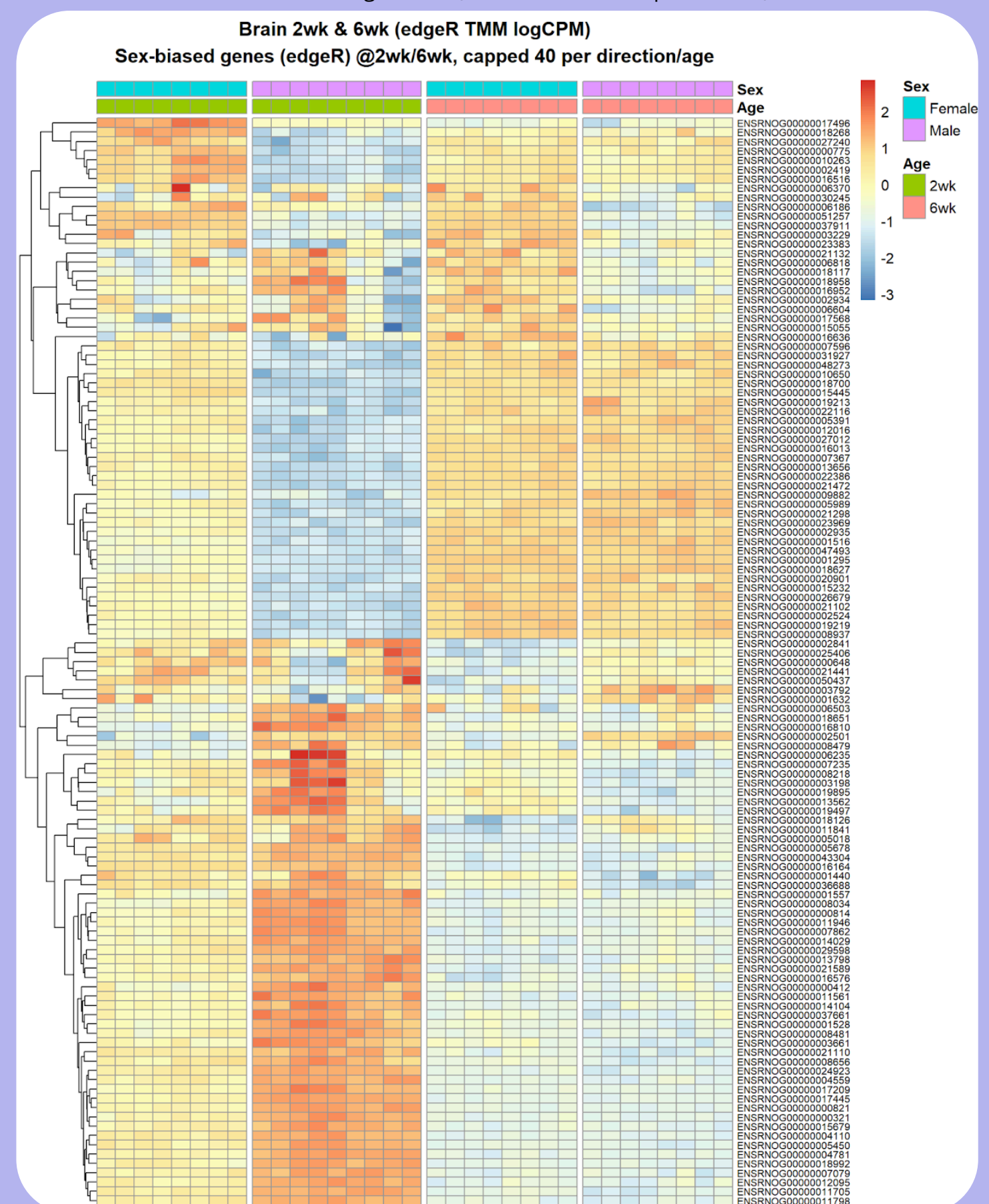


Figure 4. Heatmap of sex-biased genes in the brain at 2 and 6 weeks. Each column is one sample, annotated by Age (2 wk vs 6 wk) and Sex (Male vs Female). Rows are selected sex-biased genes (edgeR TMM, FDR < 0.05). Colors represent relative expression (z-scored per gene). Clear male-female separation is visible at 2 weeks, but weaker at 6 weeks, consistent with reduced sex-biased expression over development.