A Transcriptomic Analysis of the Estrous Cycle in 4 Regions of the Mouse Brain

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Abstract

For many years biomedical research, and in particular neuroscience research, has often focused on male subjects. Female subjects have frequently been excluded due to the perceived complications of the hormonal changes across the estrous cycle and the potential need to include the appropriate control groups. We utilized transcriptomic analysis of the hypothalamus, hippocampus, neocortex, and cerebellum of female C57BL/6J (B6) mice to examine changes in gene expression in the female mouse brain. The changes in gene expression between brain regions (n=12/brain region) and changes in gene expression within each brain region as a result of the estrous cycle (n=3/stage/tissue) were performed using the same animals. Not surprisingly, there are ~10,000 differentially expressed genes (DEGs) between the hypothalamus, hippocampus, neocortex, and cerebellum at a false discovery rate (FDR) less than 0.05. The hippocampus vs. cerebellum (n=10,610) and neocortex vs. cerebellum (n=10,464) comparisons have the most DEGs and the hippocampus vs. neocortex (n=9,166) comparison has the least. In contrast to the ~10,000 DEGs between brain regions, within each brain region there are fewer than 70 stage-specific DEGs (FDR<0.05) as a result of the estrous cycle. The hippocampus has the most DEGs (n=67), followed by the neocortex (n=55), hypothalamus (n=53), and cerebellum (n=20). Genes encoding hormones or hormone precursors that are significant DEGs in only the hypothalamus are potential candidates to be the source of changes in downstream gene expression. Six genes in the brain region-specific comparisons (*Oxt, Pomc, Esr1, Ghrh, Hcrt, Trh*) and five genes in the stage-specific comparisons (*Oxt, Hcrt, Gh, Prl, Pitx2*) fulfill these criteria. The interactions of potential candidate genes on downstream processes within the hypothalamus and between the 4 brain regions are part of ongoing analyses. This dataset demonstrates that the differences between brain regions overwhelm changes in gene expression as a result of the estrous cycle. We expect that our results will be a useful guide for researchers in the field of neuroscience in incorporating females in future experiments as well as shedding light on the interactions of hormones and gene expression in different brain regions.

Methods

Animals and Estrous Cycle Determination: All C57BL/6J (B6) mice were purchased from the Jackson Laboratories (Bar Harbor, ME) at approximately 70 days of age. After an acclimation period of two weeks the mice were euthanized and the hypothalamus, hippocampus, neocortex, and cerebellum were dissected and frozen in liquid nitrogen. Vaginal lavage was performed to determine the stage of the estrous cycle (Caligioni, 2009; Bayers et al., 2012).

RNA Sample Preparation: RNA was isolated using TriReagent (Sigma) and Next generation sequencing libraries were prepared using the NEBNext Ultra RNA Library Prep Kit for Illumina (NEB #E7530, New England Biolabs Inc., Ipswich, MA). Libraries were multiplexed, nine per flow cell lane, and were sequenced on an Illumina HiSeq 2500 (Translational Science Laboratory, College of Medicine, Florida State University). For each condition, three NGS libraries from separate animals (3 biological replicates) were sequenced. We generated up to 150 million sequencing reads per sample from a 100 base single-end sequencing run.

RNA-Seq Data Analysis: The sequencing reads were aligned using Tophat, version 2.0.8b (Trapnell et al., 2009). The *Mus musculus* genome (release 68) and linked annotation files were obtained from the Ensembl website (http://www.ensembl.org/info/data/ftp/index.html). Cufflinks version 2.1.1 (Trapnell et al., 2010) was used to generate normalized count information (FPKM values). The statistical package DESeq (Anders and Huber, 2010) was used to determine differentially expressed genes (DEGs) and an FDR-correction was used to account for multiple testing and false positives.

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5. Trapnell C, Williams BA, Pertea G, Mortazavi A, Kwan G, van Baren MJ, et al. Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. Nature biotechnology. 2010;28(5):511-5. PMID: 20436464.

Gene Expression changes across the estrous cycle

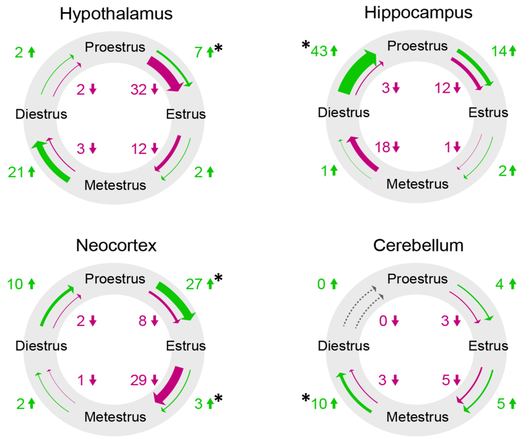

Figure 1) The number of DEG in the hypothalamus (53 genes), hippocampus (67 genes), neocortex (55 genes) and cerebellum (20 genes) across the 4 stages of the estrous cycle. For each brain region, the number of differentially expressed genes that have increased expression (green) or decreased expression (purple) across the estrous cycle. The thickness of the arrow is proportional to the number of genes and the asterisk indicates the stage transition with the largest number of DEGs for each brain region.

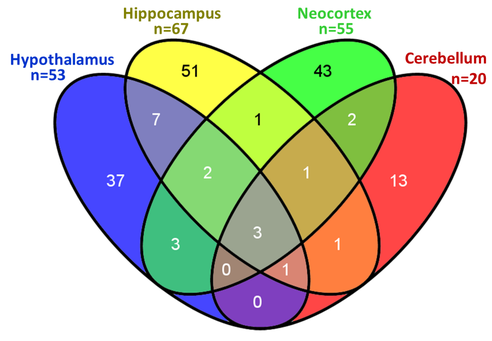

Figure 2) Venn diagram of the estrous cycle-specific DEGs in the 4 brain regions.

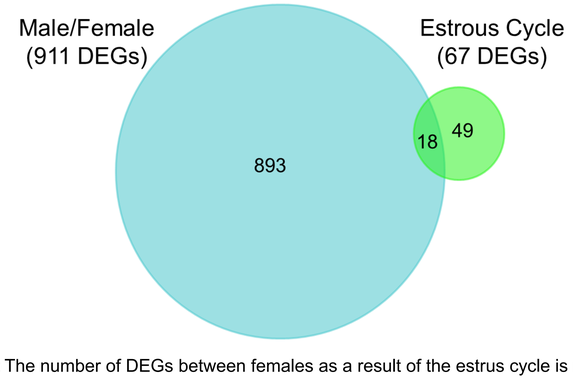

Figure 3) The number of DEGs between females as a result of the estrus cycle is much less than the number of DEGs between males and females. In the hippocampus, there are 67 DEGs as a result of the estrous cycle and 911 DEGs between male and female B6 mice.

Gene expression changes across the estrous cycle

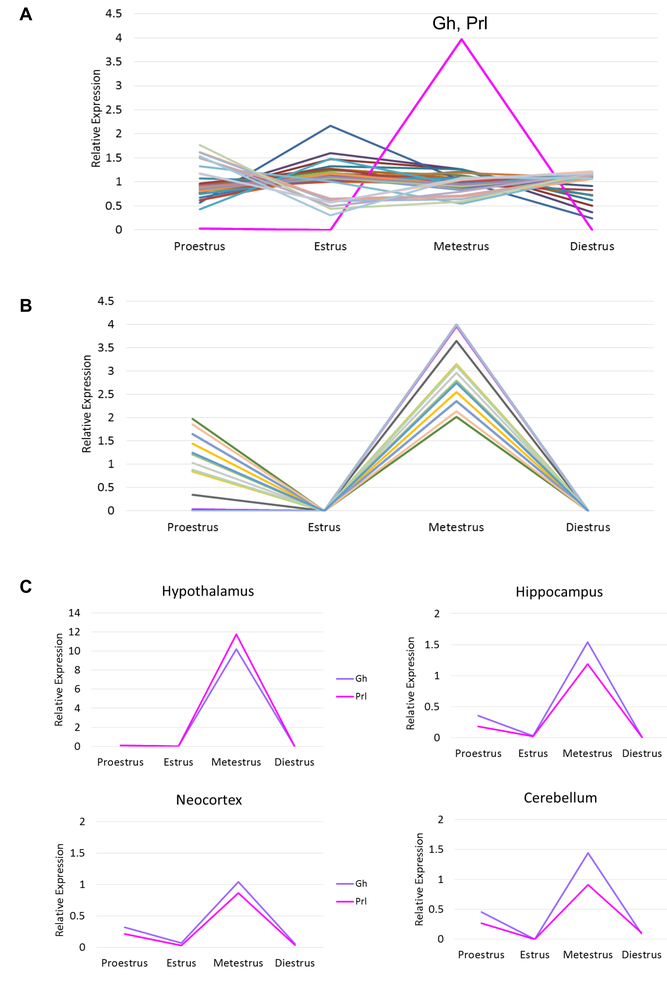

Figure 4) Growth hormone (Gh) and Prolactin (Prl) are significantly differentially expressed in the hypothalamus. A) The expression of all 53 DEGs in the hypothalamus. Gh and Prl are only expressed during metestrus. B) 55 genes that are not differentially expressed in the hypothalamus also have peak expression during metestrus and are not expressed during estrus and diestrus. C) Similarity of the expression of Gh and Prl in the hippocampus, neocortex, and cerebellum.

Conclusions

- There are DEGs across all stages of the estrous cycle in the hypothalamus, hippocampus, neocortex, and cerebellum.
- <1% of the genes in the female brain are differentially expressed as a result of the estrous cycle
- The number of DEGs see as a result of the estrous cycle are much less than the number of DEGs seen between males and females.