The Effects of Early Life Stress on Altered Gene Expression

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Abstract

Childhood trauma increases the lifetime risk of depression, drug addiction, and other mood disorders. Previous studies have suggested that the brain is sensitive to environmental factors such as stress during early development, linking early life stress (ELS) to increased sensitivity to future stress. The molecular mechanisms driving this effect are still unknown. This project focuses on one of three approaches that the lab is addressing by investigating how ELS increases sensitivity to stress on a transcriptional level. Previous RNA-seq has confirmed that ELS does result in unique transcriptional patterns across the brain's reward circuitry: ventral tegmental area (VTA), nucleus accumbens (NAc), and prefrontal cortex (PFC). This project is a follow up to the previous paper and aims to revalidate the findings. The RNA-seq broadly identified all transcriptional changes due to ELS including those on immediate early genes (IEGs), stress-related genes, and noncoding genes. The qPCR used in this project allows for gene expression analysis for specific target genes that we deemed to be more significant as the selected genes had the most significant results in the previous paper or were related to stress response. The qPCR revealed that *Grin3a* is downregulated in female ELS NAc, *Chrna6* is upregulated in male ELS NAc, and *Sgk1* is downregulated in male ELS VTA. Some of the genes that were expected to have significant expression changes did not while some of the others that did exhibit significant results had changes in opposite directions from those found in the original paper. RNA was extracted from tissue samples that were from different cohorts of adult mice, so it was very difficult to reproduce exact results. Although these results were different from the initial findings, they were sufficient to support the hypothesis that ELS results in broad transcriptional patterns.

Project Context and Rationale

Childhood trauma increases the lifetime risk of depression, drug addiction and other mood disorders[1]. Previous studies have suggested that the brain is sensitive to environmental factors such as stress during early development, linking early life stress (ELS) to increased susceptibility to future stress[2,3]. More than one million children in the US suffer some sort of traumatic experience every year. This research is crucial to better understand the neurobiological impacts of early life stress and to increase awareness about child vulnerability. Some real world implications that are currently being investigated by lab personnel include the consequences of mistreating LGBTQ children and separating children from their families in detention centers. Investigation of long-term effects would encourage improved treatment towards children during sensitive periods of development.

While ELS is known to increase the risk of depression-like behaviors later in life after experiencing adult stress, the cellular mechanisms responsible for enhanced stress susceptibility and vulnerability to depression have not yet been investigated. The lab plans to address this question by supplementing behavioral tests with three approaches: transcriptional, cellular microcircuit, and epigenetic. This project focuses on the first transcriptional approach.

We are employing a 2-hit mouse model by inducing the first hit of stress early in pup development and a second hit of stress during adulthood. We have confirmed that there are significant differences in the transcriptional patterns between standard-reared mice and ELS mice[4]. The long-lasting effects of ELS are most noticeable in the ventral tegmental area (VTA), nucleus accumbens (NAc), and prefrontal cortex (PFC) all of which play important roles in the brain's reward circuitry[4]. RNA-seq has helped identify genes within these regions with altered

expression rates including *Otx2*, which codes for a transcription factor that mediates transcription in the VTA[5]. The transcriptional approach is important for confirming that ELS correlates with cellular changes. Altered gene expression in the VTA, NAc, and PFC provides greater insight into the effects of ELS on a genetic level, supplementing previous findings of the behavioral effects of ELS.

Because of increased variability in female mice due to the estrus cycle, many previous studies involving mouse models have focused solely on working with male mice. However, since there are substantial sex differences in human depression and stressed mice[4], we included female mice in the study. GO analysis in the previous transcriptional study has suggested that pathways corresponding to neuronal outgrowth and synapse formation and signaling were enriched in both sexes[4]. Both sexes exhibited depression-like behavior and similar transcriptional changes after ELS, which further highlights the role of ELS in increased stress sensitivity.

I am conducting qPCR to identify the direction and significance of gene expression changes in genes whose products play key roles in the brain reward circuitry. My work involves revalidating the findings from prior experiments by analyzing gene expression in a new cohort of mice and determining whether the directionality of gene expression changes in the VTA, NAc, and VTA is similar or not. The previous paper used RNA-seq to broadly identify any sign of transcriptional changes due to ELS including immediate early genes (IEGs), stress-related genes, and noncoding genes. qPCR allows me to target specific genes and check the directionality and significance of those expression changes. For the purpose of the project, I investigated coding genes that had the most significant expression changes in the previous paper or genes that were

related to stress-response. Replicating the results from previous research would provide greater support for the effects of ELS on transcriptional patterns.

Methodology

RNA extraction and cDNA conversion:

Bilateral punches of VTA, NAc, and PFC tissue were collected from C57BL/6J male and female mice from both the ELS and standard-reared groups. RNA samples were purified and extracted from the tissues with RNeasy Micro Kits (Qiagen). All usable samples were confirmed to have A260/280 values ≥1.8 (Nanodrop). RNA samples were stored at -80°C until conversion into cDNA. The corresponding cDNA was created (Thermocycler) and stored at -20°C until further use.

qPCR:

Primers were designed based on the corresponding genes that were previously found to have different transcriptional patterns in response to ELS. All primers were tested with sample cDNA through Real-time PCR or qpCR in order to confirm efficiency of 80-105%; slight deviations from that efficiency range were accounted for in the calculations. Amplification plots were used to confirm that the primers were properly amplifying the cDNA, and melt curve plots were used to determine if the primers were binding to other regions besides the promoters for the target genes. Primers that had poor melt curve plots, despite being shown as within the efficiency range, were excluded from use in qPCR. cDNA created in the previous steps was used in subsequent qPCR with tested primers. Results were compared to those from Hprt1, a housekeeping gene acting as a frame of reference. Significance and directionality of

transcriptional differences were compared to the findings from the previous study to revalidate the findings.

Results

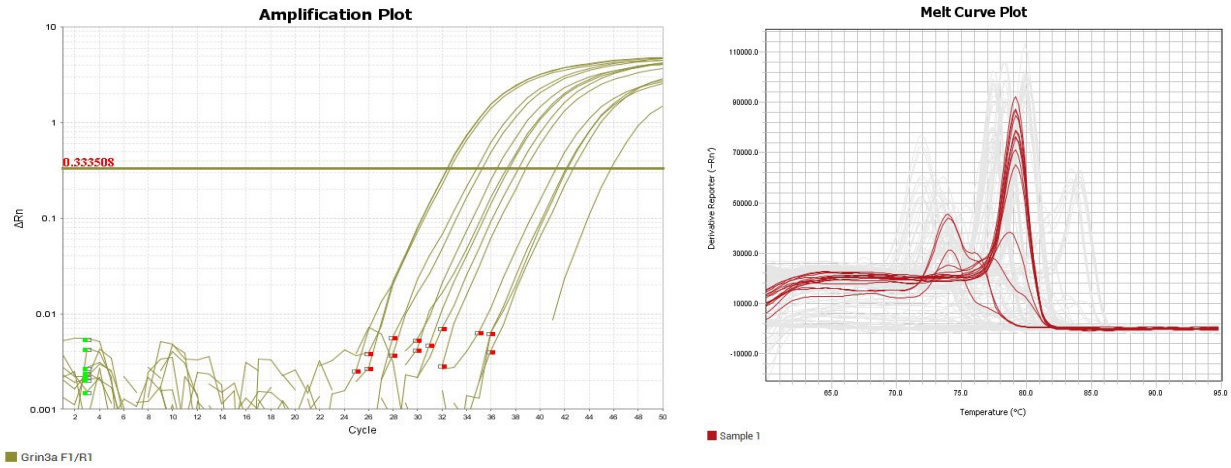

Fig 1. Examples of amplification plots and melt curve plots used to determine efficient primers.

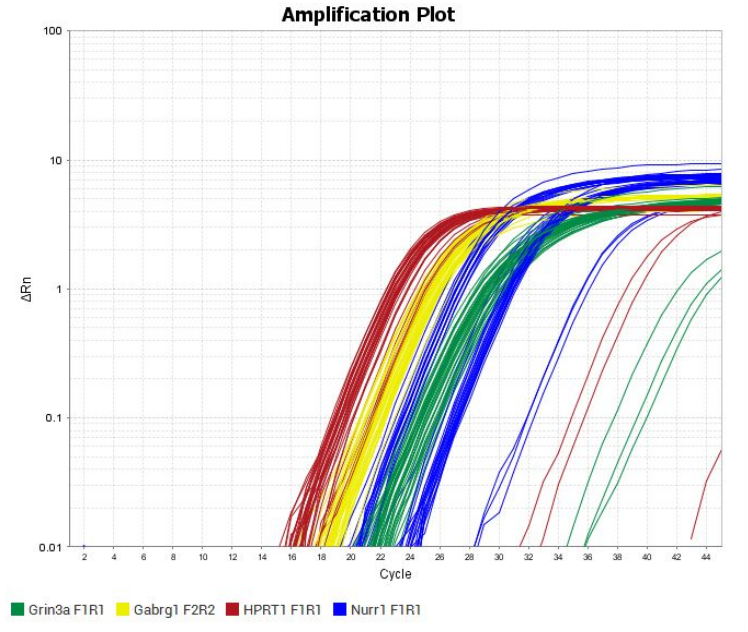

Fig 2. Amplification plot for a qPCR plate.

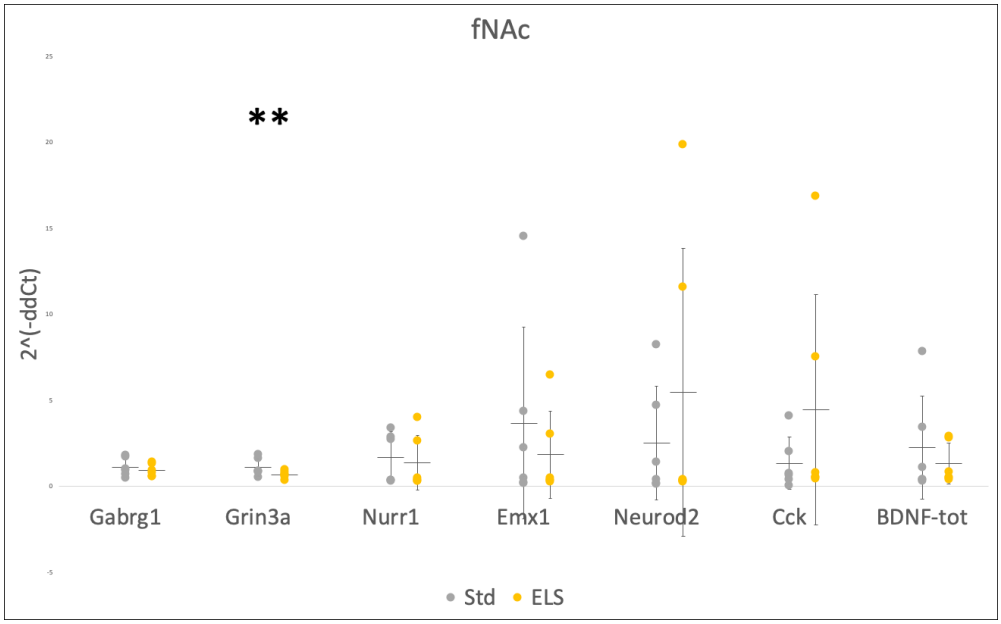

Fig 3. Relative gene expression in female NAc, Std vs. ELS; *Grin3a* has a trend of downregulation in ELS. **p<0.1

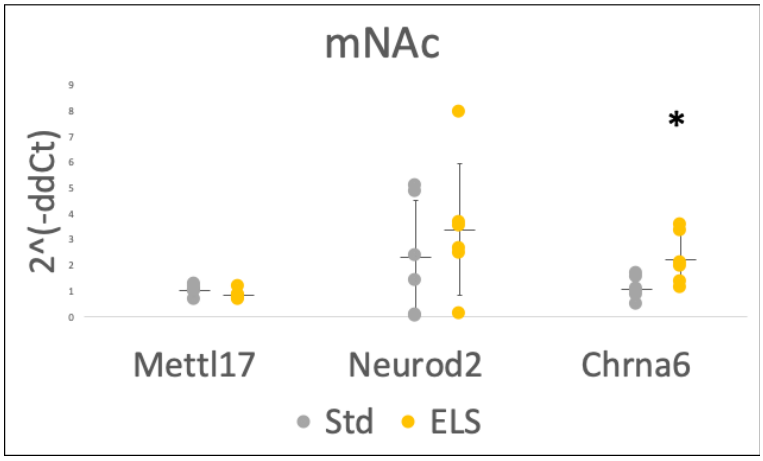

Fig 4. Relative gene expression in male NAc, Std vs. ELS; *Chrna6* is upregulated in ELS.*p<0.05

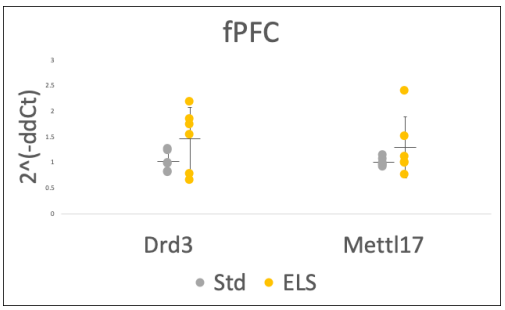

Fig 5. Relative gene expression in female NAc, Std vs. ELS. No significant altered gene expression in the target genes.

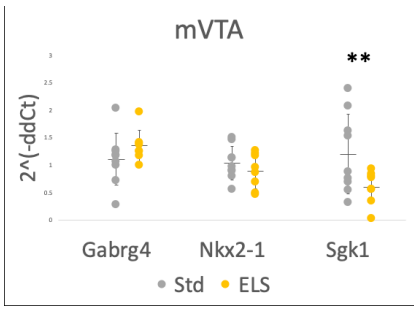

Fig 6. Relative gene expression in male VTA, Std vs. ELS; *Sgk1* is downregulated in ELS.**p<0.1

I used a fold change to represent gene expression and compared the results between the Std and ELS groups. In female NAc, *Grin3a* was downregulated for ELS (**Figure 3**, Std mean=1.094, ELS mean=0.683, p=0.099). In male NAc, *Chrna6* was upregulated for ELS (**Figure 4**, Std mean=1.09, ELS mean=2.24, p=0.029). There was no significant altered gene expression for the target genes in female PFC (**Figure 5**). In male VTA, *Sgk1* was downregulated for ELS (**Figure 6**, Std mean=1.202, ELS mean=0.605, p=0.093).

Conclusions

I still need to finish conducting qPCR for male PFC and female VTA tissue, but the results here support the hypothesis that ELS results in unique transcriptional patterns in the brain's reward circuitry. Not all of the genes that we expected to have expression changes actually had significant changes. Some of the genes that had significant results also exhibited changes in the opposite direction from those found previously. Since the tissues were from different cohorts, it was more difficult to reproduce the results. For the purpose of the project, I was attempting to reproduce the broad patterns of transcriptional changes rather than changes in specific target genes, so the results I observed here were sufficient enough to act as a follow up to the original findings. This project was able to complete the research necessary for the transcriptional level of the lab's overall aims, so future directions would be investigating ELS-activated cells and exploring histone or chromatin modifications for epigenetic studies.

References

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