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Transcriptomic signature of early life stress in male rat prefrontal cortex

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ABSTRACT

Early life stress (ELS) is associated with adverse mental health outcomes including anxiety, depression and addiction-like behaviours. While ELS is known to affect the developing brain, leading to increased stress responsiveness and increased glucocorticoid levels, the molecular mechanisms underlying the detrimental effects of ELS remain incompletely characterised.

Rodent models have been instrumental in beginning to uncover the molecular and cellular underpinnings of ELS. Limited nesting (LN), an ELS behavioural paradigm with significant improvements over maternal separation, mimics human maternal neglect.

We have previously shown that LN leads to an increase in one of the behavioural measures of anxiety like-behaviours in rats (percent of entries in the EPM open arm). Here we assessed gene expression changes induced by ELS in rat prefrontal cortex by RNA-sequencing. We show that LN leads primarily to transcriptional repression and identify a molecular signature of LN in rat PFC that is observed across ELS protocols and replicable across rodent species (mouse and rat).

1. Introduction

Early life stress (ELS) refers to any form of stress; physical, emotional or environmental changes experienced during early postnatal development (Heim and Nemeroff, 2001). ELS exposure is detrimental to the developing brain as it induces structural and gene expression alterations, as evident in animal and human (Lupien et al., 2009; Ivy et al., 2010) studies. ELS has significant impacts on emotional, social, behavioural and cognitive functions across species (Carr et al., 2013; Benetti et al., 2009; Walker et al., 2017; McLaughlin et al., 2015), and is also known to be associated with prolonged elevated glucocorticoids (Lupien et al., 2009) and increased stress responsiveness (Peña et al., 2019; Maniam et al., 2016).

In humans, it has been found that children raised in orphanages have mild cognitive impairment and social deficits (Chugani et al., 2001), exhibiting poorer spatial and long-term memory (Merz et al., 2013). They are also at an increased risk of reduced emotional control later in life (Tottenham et al., 2010) and have a greater susceptibility to mental health issues such as depression, anxiety, personality disorders and addiction (Pechtel and Pizzagalli, 2011; Enoch, 2011). Children who have experienced chronic ELS have been observed to have altered brain volume (Mehta et al., 2009), including reduced prefrontal cortex (PFC) and hippocampal volume and enlarged amygdala (Tottenham et al., 2010; Frodl et al., 2010).

In rats, ELS induced by maternal separation led to anxiety-like behaviour and hyperactivity of the stress system (Maniam and Morris, 2010). More recently, limited nesting (LN), which involves dams being supplied with inadequate nesting materials, has been shown to mimic the behavioural patterns of maternal neglect in humans (Walker et al., 2017; Ivy et al., 2008). We previously reported in rats that ELS induced by LN from postnatal days 2–9 led to increased anxiety-like behaviour of offspring, as measured by an increase in the % entries in the EPM open arm (Maniam et al., 2016), in line with other studies employing the LN model (Walker et al., 2017). We also found that within the hippocampus, LN led to altered expression of genes implicated in plasticity and mood regulation, as well as *Akt 3* mRNA, a key gene involved post-natal brain development (Maniam et al., 2016).

Here we aimed to examine gene expression changes in the PFC, a

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brain region also implicated in stress responses and mood regulation (Urb et al., 2019). Dysregulation of the PFC-amygdala circuit is one of the core neurobiological features of stress-induced anxiety in humans and animal models (Hultman et al., 2016; Murray et al., 2011; Price and Drevets, 2012; Robinson et al., 2014; Shin et al., 2005). This circuit appears to be particularly sensitive to ELS (VanTieghem and Tottenham, 2018). ELS alters the neurodevelopmental apoptosis of neurons as well as early-postnatal proliferation and apoptosis of glial cells in PFC (Majcher-Maslanka et al., 2019). ELS also impairs the emergence of amygdala projections to the PFC (Ishikawa et al., 2015), resulting in altered amygdala-prefrontal circuit function and anxiety-like behaviors in adult rats (Ishikawa et al., 2015). Furthermore, ELS perturbs the functional maturation of PFC-hippocampal networks (Reincke and Hanganu-Opatz, 2017). While accumulating evidence demonstrates that ELS alters PFC function, the underlying molecular mechanisms are less explored.

Thus we carried out the first genome-wide assessment of gene expression changes in rat PFC as a result of LN-induced ELS. To further examine whether ELS induces a robust gene expression signature in PFC across species, we conducted a detailed comparison with recent data in the same brain region from mice subjected to a different ELS paradigm (Peña et al., 2019).

2. Methods

- Limited nesting

All procedures were approved by the Animal Care and Ethics Committee of UNSW. Male and Sprague-Dawley rats (Animal Resource Centre, Perth, WA, Australia) were maintained in a temperature controlled (23 °C) colony room on a 12 h light/dark cycle (lights on at 0700) with *ad libitum* access to standard laboratory chow and water. Mating was carried out in house to generate litters. After mating females were housed singly and littered normally. From post-natal days 2–9 the ELS cages had a metal grid floor inserted that allowed waste collection and provided only half a sheet of paper towel as nesting material. ELS rats were returned to normal bedding at PND 10. The control group had normal bedding throughout. At PND 21, pups were weaned and housed 3–4 rats per cage. Brain tissues were collected from male offspring at 13 weeks after terminal anaesthesia (Ketamine/Xylazine) and decapitation (Fig. 1). This cohort formed part of an earlier study describing hippocampal changes (Maniam et al., 2016). Across both the ELS and control groups 5 offspring were sampled from 4 different mothers. The 5 offspring were chosen so that the litter composition of the two groups was identical (Supplementary Table 1) to avoid litter-dependent effects.

- Anxiety-like behavioural testing

Anxiety-like behaviour was assessed at 10 weeks of age as previously described (Maniam et al., 2016) using elevated plus maze (EPM), a plus shaped maze consisting of two open and two closed arms. Rats were placed in the EPM for 10 min; the time spent and entries into each of the open and closed arms was recorded. The % entries in the open arm was

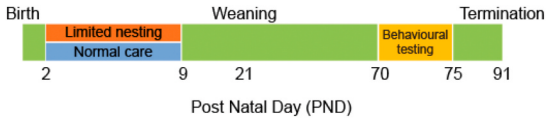

Fig. 1. Schematic depiction of experimental design. Dams gave birth and from postnatal (PND) 2 to 9, litters were exposed to either normal nesting (control, blue) or limited nesting (ELS, red) conditions. All pups were weaned onto normal chow at PND21, and behavioural testing was carried out at 10 weeks of age. Rats were euthanised at 13 weeks for brain tissue collection. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

significantly different between the ELS and control groups (n = 7–10 per group), with the ELS rats making ~20% less open arm entries compared to controls ($p < 0.05$) (Maniam et al., 2016). The percentage of time spent in open arms showed a similar trend, but did not reach statistical significance (Maniam et al., 2016). There were no significant differences across groups in general activity (i.e. number of entries into open arms), or in spatial and object recognition memory tests (i.e. novel object and place recognition tasks) (Maniam et al., 2016).

We confirmed that the difference in the % entries in the open arm remained significantly different between the ELS and control groups for the subset of 5 offspring selected for RNA extractions (Supplementary Table 1 %OpenArmEntries; $p = 0.03$, Wilcoxon rank sum test).

- RNA extraction

Tissue collection was carried out at post-natal day 91. RNA was extracted from 5 male rat PFC tissue samples per group using the Qiagen miRNeasy Mini kit (#217004) with on-column DNase digestion using the QIAGEN RNase-Free DNase kit (#79254) according to the manufacturers protocol. RNA and DNA concentrations in each sample were assessed using a Qubit RNA BR Assay Kit (Q10210) and a Qubit dsDNA HS Assay Kit (#Q32851). RNA quality was assessed using an Agilent Bioanalyzer at the UNSW Ramaciotti Centre for Genomics. The RNA and DNA concentrations as well as the RNA Integrity Numbers (RIN) are listed in Supplementary Table 1.

- RNA-sequencing

RNA-seq library preparation and Illumina RNA-sequencing were carried out at the UNSW Ramaciotti Centre for Genomics. Library preparation was carried out on 3 μg total RNA used as input for polyA selection and library preparation using an Illumina TruSeq Stranded mRNA-seq kit. Libraries were barcoded and sequenced on one NextSeq 500 run, to obtain an average of 23.5 million 75bp paired-end reads per sample (Supplementary Table 2).

RNA-seq reads were mapped to the rat genome rn6 using the STAR spliced aligner (Dobin et al., 2013) with the following parameters: -outSJfilterOverhangMin 5 5 5 5 –alignSJoverhangMin 5 –alignSJDBoverhangMin 5 –outFilterMultimapNmax 1 –outFilterScoreMin 1 –outFilterMatchNmin 1 –outFilterMismatchNmax 2 –chimSegmentMin 5 –chimScoreMin 15 –chimScoreSeparation 10 –chimJunctionOverhangMin 5. Data quality was assessed using *Qualimap* (Okonechnikov et al., 2016) (quality metrics are listed in Supplementary Table 2). Gene expression quantification was carried out using the *featureCounts* function as implementer in STAR using the Ensembl rat genome annotation, release 89.

Genes were filtered to include those expressed at a minimum of 0.5 reads per kilobase per million (rpkm) in a minimum of 2 samples, i.e. 16,041 genes. Differential expression analysis contrasting ELS and control samples was carried out using a generalised linear model with a negative binomial Wald test as implemented in the *DEseq* function in the DESeq2 R package (Love et al., 2014). p-values were corrected for multiple testing using the Benjamini-Hochberg correction. Genes were considered differentially expressed if adjusted p-value <0.05 and absolute fold-change >1.3.

- Microarrays

Gene expression quantification on Affymetrix Clariom_S_Rat arrays was carried out at the UNSW Ramaciotti Centre for Genomics using 500 ng of the same total RNA sample used for RNA-seq. Data import from. CEL files was performed in R using the Bioconductor *oligo* package (Carvalho and Irizarry, 2010) and *pd. clariom.s.rat* (MacDonald, 2016). Probe annotation to gene symbols was obtained from Affymetrix (Clariom_S_Rat.na36. rn6. transcript.csv, www.affymetrix.com/Auth/analysis/downloads/na36/xta/Clariom_S_Rat.na36.rn6.transcrip

t.csv.zip). Microarray data was quantile normalised and log2-transformed using the *rma* function in the *Affy* Bioconductor R package. Differential expression analysis was carried out using a liner model as implemented in the *lmFit* function in the *limma* R package.

- *In-silico* deconvolution

Cellular composition was estimated using Decon RNA-seq (Gong and Szustakowski, 2013) with default parameters. DeconRNA-seq is a gene expression deconvolution method that uses a Non-negative Least Squares approach to estimate the proportion of cell types in bulk RNA transcriptome data. It requires as input the bulk RNA-seq data (X) and a matrix of gene expression in pure cell types (S). The output is a vector (P) of cell type proportions for each sample, obtained by optimizing X ~ S*P. Cell type proportions for each sample are constrained to sum to one. Reference RNA-seq data (S) was obtained from mouse neurons, astrocytes, oligodendrocytes, microglia and endothelial cells from Zhang et al. (2014).

- Other statistical analyses

Gene ontology enrichment analysis was carried out using gProfiler (Reimand et al., 2007) with the 16,041 expressed genes as background. P-values were corrected for multiple testing using the Benjamini-Hochberg correction.

To test how well the DE results from Peña et al. (2019) are replicated in our data we used a rank-rank hypergeometric overlap test, as implemented in the RRHO Bioconductor package. The two ranked lists consisted of the DE genes from Pena et al. ranked by either the DE data from Pena et al. or the DE data from our study. Genes were ranked by -log 10 (p-value) multiplied by the sign of the fold change, to take into account the direction of gene expression change. The rank-rank hypergeometric overlap was calculated using the *RRHO* function, with alternative = two-tailed and all other parameters as default. The significance p-value was determined by resampling using the *pvalRRHO* function with 10^6 replications.

Standard hypergeometric overlap test was carried out using the *phyper* R function.

The correlation between gene expression and %Open Arm Entries was assessed using Pearson correlation coefficients and the p-value was obtained using the *cor. test* function in R.

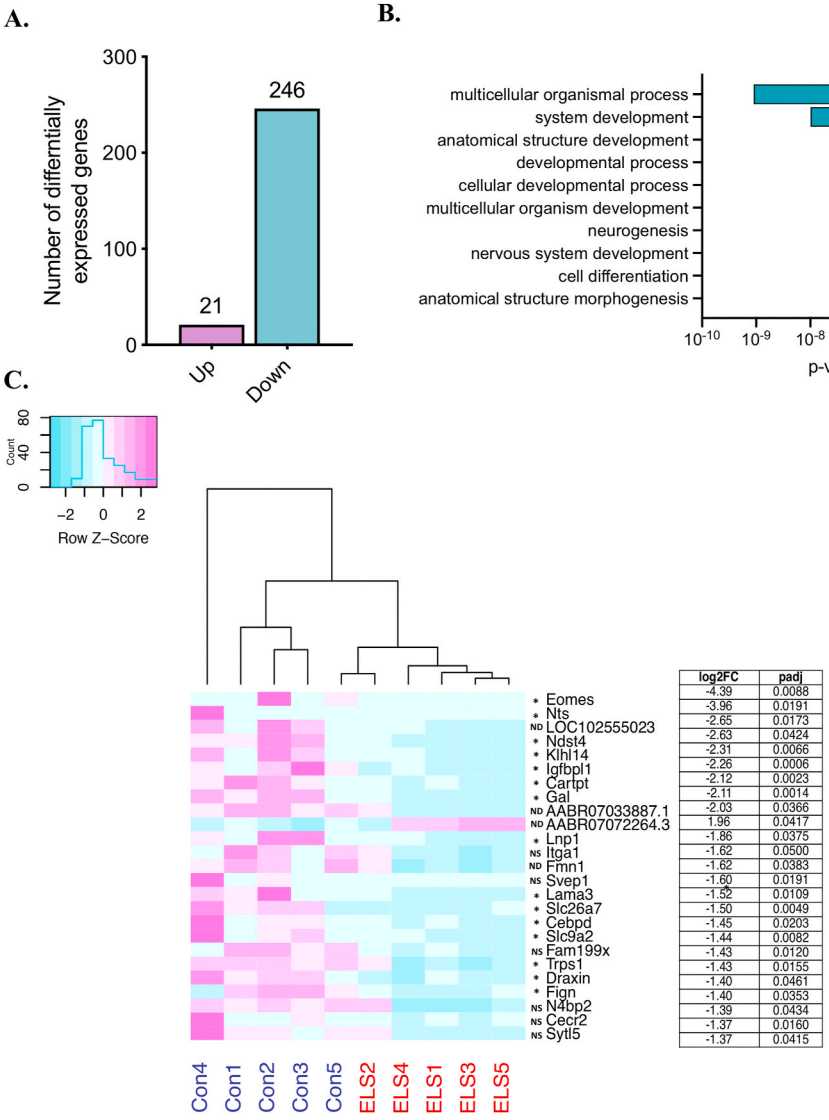

Fig. 2. Gene expression changes in rat prefrontal cortex in response to ELS. **A.** Barplot of the number of up- and down-regulated genes. **B.** Selected gene ontology terms enriched in DE genes. *x-axis:* multiple-testing corrected enrichment p-values. The full list of enriched GO terms is provided in Supplementary Table 4. **C.** Heatmap of scaled gene expression values for the top differentially expressed genes (left) and a table of log2 fold-changes and adjusted p-values (right). Labels on the left of gene symbols designate significance of differential expression in the microarray data: *-significantly DE and in the same direction of change as in the RNA-seq data; NS- non-significant; ND-not detected on microarrays.

3. Results

3.1. ELS leads to gene expression down-regulation in prefrontal cortex

We have previously shown that LN in this early post-natal window (P2–P9) leads to anxiety-like behaviours in male rats (Maniam et al., 2016). To investigate whether the anxiety-like behaviour induced by LN is associated with transcriptome changes in PFC, we carried out RNA-seq of PFC samples from 5 ELS and 5 Control male rats from the cohort used for behavioural testing (Methods; Supplementary Tables 1 and 2). We identified 276 differentially expressed (DE) genes (p-value adjusted for multiple testing < 0.05, absolute fold change > 1.3). Notably, the vast majority of DE genes (92%, 245 genes) were downregulated in response to ELS (Fig. 2; Supplementary Table 3), suggesting that LN was associated with transcriptional repression. The down-regulated genes were strongly enriched for genes involved in nervous system development, neurogenesis, and neuronal differentiation (Fig. 2 and Supplementary Table 4), and the top down-regulated gene was *Eomes/Tbr2*, a transcription factor important for neurogenesis and neuronal differentiation (Mihalas and Hevner, 2017; Lv et al., 2019). Galanin (*Gal*) was also down regulated (Supplementary Table 3). This gene encodes a neuropeptide implicated in mood disorders in humans (Barde et al., 2016) while rat *Gal* was postulated to promote stress-resilient neuroplasticity (Hooversmith et al., 2019). Upregulated genes did not show any significant gene ontology term enrichment; the top most significantly upregulated genes were the transcriptional repressor *Fezf2* and the transmembrane protein *Tmem178a* (Supplementary Table 4).

To assess whether these gene expression differences could reflect differences in cell-type composition, we estimated the cellular composition of each sample using *in-silico* deconvolution (Methods). We found that the cellular composition of the ELS and Control samples were similar, with differences of less than 10% for any cell type (Fig. 3). Since such small differences cannot account for gene expression changes of more than 30% (i.e. 1.3 fold-change used as threshold), the observed gene expression differences are consistent with transcriptional dysregulation rather than differences in cell type composition.

We also quantified gene expression in the 5 ELS and 5 Control PFC samples using Affymetrix microarrays for technical validation (Methods, Supplementary Fig. 1, Supplementary Table 3). Of the 267 DE genes identified by RNA-seq, 245 were detected as expressed by microarrays and of these 210 genes showed expression changes in the same direction as in the RNA-seq data (Supplementary Fig. 1).

We have previously shown that LN-induced ELS leads to increased anxiety in male offspring as reflected by reduced % entries in the open arm of the EPM (Maniam et al., 2016). In addition, recent data demonstrated that the control exerted by PFC over amygdala is altered by stress, resulting in an anxiety phenotype associated with changes in EPM behaviour (Liu et al., 2020). Thus we assessed the correlation between gene expression in PFC and % entries in the EPM open arm, as well as % time spent in the EPM open arm. For the rats included in the current study, % entries in the open arm (Supplementary Table 1) was significantly lower in the ELS group ($p = 0.03$ Wilcoxon rank sum test) while the % time spent in the open arm (Supplementary Table 1) did not reach significance group ($p = 0.055$ Wilcoxon rank sum test). We found that down-regulated DE genes tended to show positive correlations with % entries in the open arm and % time spent in the open arm, while up-regulated DE genes showed negative correlations with both measures (Supplementary Fig. 2). A larger number of DE genes showed significant correlation with % entries in the open arm than with % time spent in the open arm (Supplementary Fig. 2). In addition, some genes were strongly correlated with % entries in the open arm but did not reach statistical significance for DE. The 12 top such genes are shown in Supplementary Fig. 3, and the correlation data for both measures and all genes is included in Supplementary Table 5.

3.2. A robust gene expression signature of ELS in prefrontal cortex

A recent study by Peña et al. (2019) assessed gene expression changes in mouse PFC in response to ELS induced at P10–P20 by a combination of LN and maternal separation. The experimental design in Peña et al. (2019) differed from the present study in terms of species (mouse and rat) and ELS paradigms. It included multiple brain regions (PFC, nucleus accumbens and ventral tegmental area), and males and females in each group.

We thus investigated whether a gene expression signature replicable across species and ELS models was observed in the PFC. To this end, we compared our DE genes with those reported by Peña et al. (2019) as DE in male ELS vs. male control PFC samples.

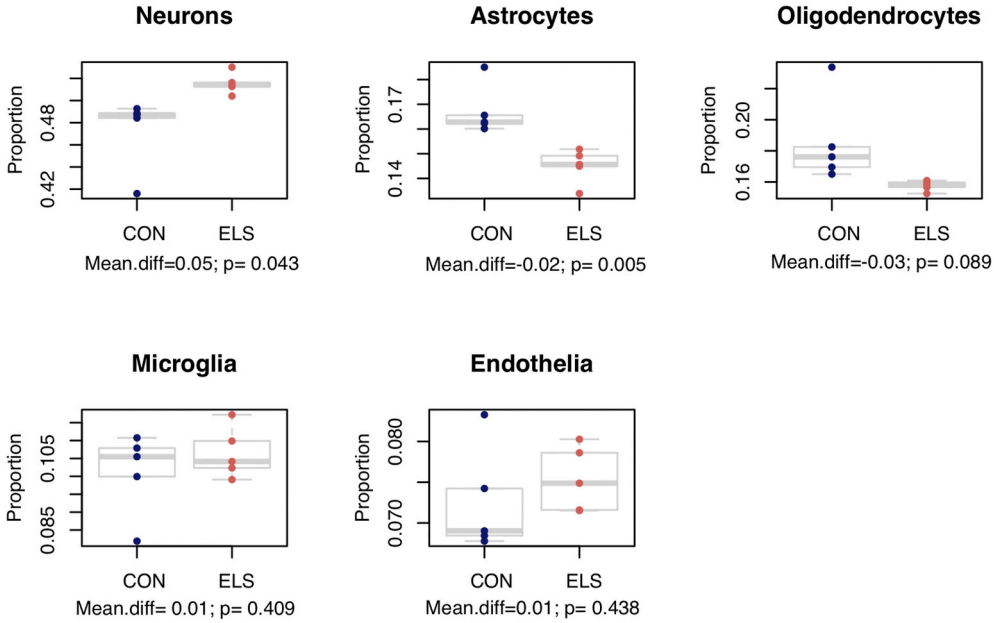

Fig. 3. Cell type proportions estimated by in-silico deconvolution. For each cell type boxplots display the distribution of proportions in the two groups; Control and ELS. Dots represent individual samples. *Mean. diff*-difference in mean proportion between ELS and control groups. *p: t-test* p-value.

We observed a highly significant overlap between the DE genes from the two studies, using either a rank-rank hypergeometric overlap test ($p = 7.9 \times 10^{-5}$; Methods), or a standard hypergeometric overlap test for genes DE at $p < 0.05$ in both studies ($p = 2.03 \times 10^{-25}$). The nominal p-value threshold was used as the Peña et al. (2019) study did not observe genes significantly DE after multiple testing correction.

Similar to what we observed in rat, the majority (84%) of the DE genes reported by Peña et al. (2019) (180 genes, $p < 0.05$) were down-regulated in response to ELS. Notably, all 40 genes DE in both studies at p- < 0.05 (Supplementary Table 6) showed gene expression changes in the same direction (Fig. 4), and 20 of them passed a multiple testing corrected $p < 0.05$ in our data. These results show that although gene expression changes induced by ELS are generally of low magnitude, a robust and replicable gene expression signature is detectable in PFC. This gene expression signature appears to be specific for male PFC as only 1 of the 40 genes was dysregulated in response to ELS in nucleus accumbens, and none of them in ventral tegmental area (VTA); Fig. 4. Moreover, only 3 of the 40 genes were dysregulated in female PFC (Peña et al., 2019); Fig. 4.

Notably, *Eomes/Tbr2* was strongly down-regulated in both our data and the data from Peña et al. (2019), highlighting a potential role for this transcription factor in transcriptional responses in ELS. The gene expression signature of ELS also included downregulation of *Draxin*, which encodes a protein important for axon guidance (Zhang et al., 2010), and *Igfbpl1* which has recently been implicated in regulating axon growth. The reduction in Igfbpl1 is of interest given its recently suggested role in axon growth (Guo et al., 2018) in the mouse.

4. Discussion

Here we carried out a genome-wide analysis of gene expression changes in response to ELS induced by LN in male rat PFC and identified a PFC-specific gene expression signature that is robust across rodent species and ELS paradigms. Accumulating evidence from multiple rodent models of ELS supports the notion that behavioural phenotypes induced by ELS are associated with epigenomic changes, which in turn leads to changes in gene expression (Torres-Berrío et al., 2019). Most of these data come from studies of hippocampus, amygdala, and the VTA (reviewed in (Torres-Berrío et al., 2019)).

The PFC is an important component of the mesocorticolimbic system that is sensitive to ELS in rodents (Yang et al., 2017; Curley and Champagne, 2016; Blaze et al., 2015). Lesions of the PFC, either ischemic (Deziel and Tasker, 2018) or pharmacologically induced (Shah and Treit, 2003), lead to an increase on the percentage of open arm entries in the EPM test, demonstrating that the PFC regulates anxiety-like behaviors reflected in EPM tests. Furthermore, presynaptic glutamate release in PFC-to-basolateral amygdala projection neurons correlates with the % open arm entries in chronically stressed mice but not in controls (Liu et al., 2020). In humans, the PFC is implicated in major depressive disorder and schizophrenia (Russo and Nestler, 2013).

We found that gene expression changes in PFC in response to ELS consisted primarily in transcriptional down-regulation, possibly suggesting epigenetic transcriptional repression. Several of the down-regulated genes are involved in neurogenesis and neuronal differentiation, but also myelination and oligodendrocyte differentiation (Supplementary Tables 3 and 4).

Extensive research done in animal models has linked ELS with altered plasticity and neurogenesis of the hippocampus (Loi et al., 2017) and little previous work has examined the PFC (Reincke and Hanganu-Opatz, 2017; Farrell et al., 2016; Rincel et al., 2018). Dendritic morphology was reduced in the medial PFC in rats exposed to ELS in the form of pup-dam separation during the early postnatal period (Rincel et al., 2018). Mild aversive early stress exposure in rats led to decreased neuronal density in the PFC as early as postnatal day 12 (Stamatakis et al., 2016). These morphological studies complement our findings on ELS induced down-regulation of genes associated with neurogenesis and neuronal differentiation. The fact that we observe reduced expression of genes involved in neurogenesis and neuronal differentiation at 13 weeks, when these cellular processes are largely completed, suggests persistent epigenetic silencing of genes involved in neurogenesis.

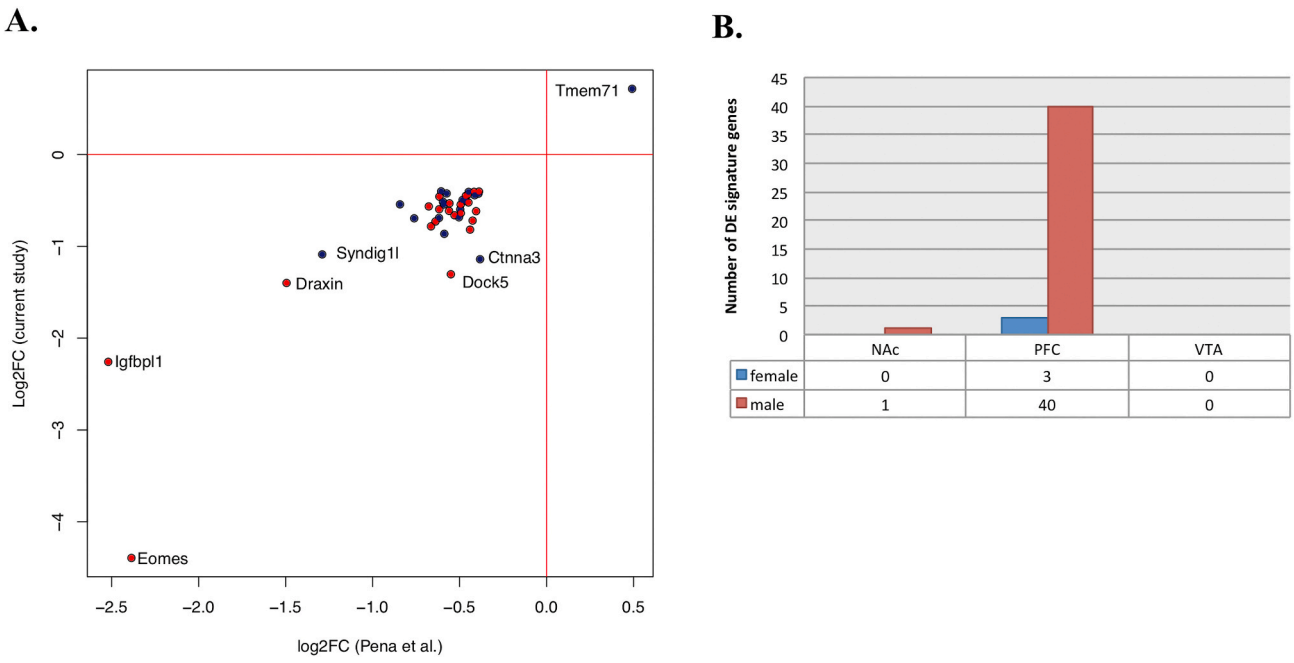

Fig. 4. Comparison of gene expression changes in response to ELS between rat and mouse. A. Scatterplot of log2 fold-changes observed in male rat PFC in the present study (*y-axis*) and those reported in male mouse PFC by Peña et al. (*x-axis*). *Blue:* genes significant at $p < 0.05$ in both studies. *Red:* genes passing the more stringent threshold of FDR <0.5 in the present study. **B.** Brain-region specificity of PFC gene expression changes in response to ELS. The bar-plot displays the number of genes identified as DE in the present study at $p < 0.05$ that were also detected as DE at $p < 0.05$ in mouse across different brain regions and sexes (Peña et al.). PFC: prefrontal cortex, NAc: nucleus accumbens, VTA: ventral tegmental area. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Stress has been previously reported to lead to complex alterations in cortical cellular composition. Impaired myelination and oligodendrogenesis has been reported in multiple rodent models of chronic stress (Birey et al., 2015; Lehmann et al., 2017; Liu et al., 2018; Yang et al., 2016). ELS induced by maternal separation was shown to impair postanatal oligodendrogenesis in the medial PFC (Teissier et al., 2020) while another study has shown that rats exposed to maternal separation had more neurons, astrocytes and NG2-glia and less microglia in the prelimbic region of the PFC (Majcher-Maslanka et al., 2019).

Our cellular composition estimates showed a higher proportion of neurons (mean difference of 5%, $p = 0.04$; Fig. 3) and lower proportion of astrocytes (mean difference of 2%, $p = 0.005$; Fig. 3) in the ELS group, while cell type proportions were not significantly different for oligodentrocytes, microglia and endothelial cells (Fig. 3).

Since our goal was to identify changes in gene expression regulation, we set the fold-change threshold at 30% (i.e. 1.3), so the DE results would not be affected by cellular composition differences. The most enriched gene ontology terms, related to neuronal function (eg. Neurogenesis, nervous system development; Fig. 2B) were enriched in the list of downregulated genes. Since the proportion of neurons was higher in ELS, these data are consistent with the notion that the observed neuronal gene expression changes did not reflect cellular composition differences. We also observed a downregulation of genes involved in myelination, axon ensheathment and oligodentrocyte differentiation. The estimated proportion of oligodendrocytes was not significantly different between groups ($p = 0.09$; Fig. 3), but showed a trend of lower proportion in the ELS group (mean difference of 3%; Fig. 3). We believe that the observed downregulation of genes involved in oligodentrocyte functions is stronger than what would be expected to result from the estimated difference in cellular composition. It is possible however that the observed differences in oligodendrocyte-specific genes partially reflect differences in oligodendrocyte numbers. Notably, stress has previously been reported to lead to both a reduction in oligodendrocyte precursor cells in PFC (Birey et al., 2015), and downregulation of myelination-related genes in the PFC (Liu et al., 2018). Further studies would be needed to directly assess the oligodendrocyte cell abundance in the PFC in response to LN.

Comparing our data with a recent study assessing gene expression changes in mouse following a combined paradigm of LN and maternal separation we found significant overlap of differentially expressed genes. While in the study of Peña et al. (2019) gene expression changes were milder, with no genes passing FDR <0.05, we found > 200 genes significantly DE at FDR<0.05 and absolute FC > 1.3. The difference might reflect species differences between mouse and rat and/or differences between ELS paradigms. The study by Peña et al. (2019) did not observe any gene expression changes when ELS occurred in the early developmental period equivalent to our study (P2–P9). However, genes reported by Peña et al. (2019) as DE at a nominal $p < 0.05$ for P10–P20 ELS showed strong overlap with those identified in our study, highlighting a robust gene expression signature of ELS in the rodent PFC.

Overall, our study provides a genome-wide characterisation of gene expression changes in response to ELS in rat PFC and demonstrates that ELS leads to replicable gene expression changes across species, primarily consisting of transcriptional repression of genes involved in neurogenesis and myelination.

CRediT authorship contribution statement

Nicole Oldham Green: Formal analysis, Investigation, Writing – original draft. **Jayanthi Maniam:** Conceptualization, Investigation, Writing – original draft. **Jessica Riese:** Investigation, Writing – original draft. **Margaret J. Morris:** Conceptualization, Resources, Writing – original draft, Supervision, Funding acquisition. **Irina Voineagu:** Conceptualization, Formal analysis, Writing – original draft, Supervision, Funding acquisition.

Declaration of competing interest

The authors have no conflict of interest to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ynstr.2021.100316.

Code availability

Data analysis code is available upon request.

References

Barde, S., et al., 2016. Alterations in the neuropeptide galanin system in major depressive disorder involve levels of transcripts, methylation, and peptide. Proc. Natl. Acad. Sci. Unit. States Am. 113 (52), E8472–E8481.
Benetti, F., et al., 2009. Early postnatal maternal deprivation in rats induces memory deficits in adult life that can be reversed by donepezil and galantamine. Int. J. Dev. Neurosci. 27 (1), 59–64.
Birey, F., et al., 2015. Genetic and stress-induced loss of NG2 glia triggers emergence of depressive-like behaviors through reduced secretion of FGF2. Neuron 88 (5), 941–956.
Blaze, J., Asok, A., Roth, T.L., 2015. Long-term effects of early-life caregiving experiences on brain-derived neurotrophic factor histone acetylation in the adult rat mPFC. Stress 18 (6), 607–615.
Carr, C.P., et al., 2013. The role of early life stress in adult psychiatric disorders: a systematic review according to childhood trauma subtypes. J. Nerv. Ment. Dis. 201 (12), 1007–1020.
Carvalho, B.S., Irizarry, R.A., 2010. A framework for oligonucleotide microarray preprocessing. Bioinformatics 26 (19), 2363–2367.
Chugani, H.T., et al., 2001. Local brain functional activity following early deprivation: a study of postinstitutionalized Romanian orphans. Neuroimage 14 (6), 1290–1301.
Curley, J.P., Champagne, F.A., 2016. Influence of maternal care on the developing brain: mechanisms, temporal dynamics and sensitive periods. Front. Neuroendocrinol. 40, 52–66.
Deziel, R.A., Tasker, R.A., 2018. Bilateral ischaemic lesions of the medial prefrontal cortex are anxiogenic in the rat. Acta Neuropsychiatr. 30 (3), 181–186.
Dobin, A., et al., 2013. STAR: ultrafast universal RNA-seq aligner. Bioinformatics 29 (1), 15–21.
Enoch, M.A., 2011. The role of early life stress as a predictor for alcohol and drug dependence. Psychopharmacology (Berlin) 214 (1), 17–31.
Farrell, M., et al., 2016. Sex-specific effects of early life stress on social interaction and prefrontal cortex dendritic morphology in young rats. Behav. Brain Res. 310, 119–125.
Frodl, T., et al., 2010. Interaction of childhood stress with hippocampus and prefrontal cortex volume reduction in major depression. J. Psychiatr. Res. 44 (13), 799–807.
Gong, T., Szustakowski, J.D., 2013. DeconRNASeq: a statistical framework for deconvolution of heterogeneous tissue samples based on mRNA-Seq data. Bioinformatics 29 (8), 1083–1085.
Guo, C., et al., 2018. IGFBPL1 regulates axon growth through IGF-1-mediated signaling cascades. Sci. Rep. 8 (1), 1–13.
Heim, C., Nemeroff, C.B., 2001. The role of childhood trauma in the neurobiology of mood and anxiety disorders: preclinical and clinical studies. Biol. Psychiatr. 49 (12), 1023–1039.
Hooversmith, J.M., Bhatti, D.L., Holmes, P.V., 2019. Galanin administration into the prelimbic cortex impairs consolidation and expression of contextual fear conditioning. Behav. Brain Res. 375, 112160.
Hultman, R., et al., 2016. Dysregulation of prefrontal cortex-mediated slow-evolving limbic dynamics drives stress-induced emotional pathology. Neuron 91 (2), 439–452.
Ishikawa, J., Nishimura, R., Ishikawa, A., 2015. Early-life stress induces anxiety-like behaviors and activity imbalances in the medial prefrontal cortex and amygdala in adult rats. Eur. J. Neurosci. 41 (4), 442–453.
Ivy, A.S., et al., 2008. Dysfunctional nurturing behavior in rat dams with limited access to nesting material: a clinically relevant model for early-life stress. Neuroscience 154 (3), 1132–1142.
Ivy, A.S., et al., 2010. Hippocampal dysfunction and cognitive impairments provoked by chronic early-life stress involve excessive activation of CRH receptors. J. Neurosci. 30 (39), 13005–13015.

Lehmann, M.L., et al., 2017. Chronic social defeat reduces myelination in the mouse medial prefrontal cortex. Sci. Rep. 7, 46548.
Liu, J., et al., 2018. Widespread transcriptional alternations in oligodendrocytes in the adult mouse brain following chronic stress. Dev Neurobiol 78 (2), 152–162.
Liu, W.Z., et al., 2020. Identification of a prefrontal cortex-to-amygdala pathway for chronic stress-induced anxiety. Nat. Commun. 11 (1), 2221.
Loi, M., et al., 2017. Effects of early-life stress on cognitive function and hippocampal structure in female rodents. Neuroscience 342, 101–119.
Love, M.I., Huber, W., Anders, S., 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 15 (12), 550.
Lupien, S.J., et al., 2009. Effects of stress throughout the lifespan on the brain, behaviour and cognition. Nat. Rev. Neurosci. 10 (6), 434–445.
Lv, X., et al., 2019. TBR2 coordinates neurogenesis expansion and precise microcircuit organization via Protocadherin 19 in the mammalian cortex. Nat. Commun. 10 (1), 1–15.
MacDonald, J.W., 2016. pd.clariom.s.rat: Platform Design Info for Affymetrix Clariom_S_Rat.
Majcher-Maslanka, I., Solarz, A., Chocyk, A., 2019. Maternal separation disturbs postnatal development of the medial prefrontal cortex and affects the number of neurons and glial cells in adolescent rats. Neuroscience 423, 131–147.
Maniam, J., Morris, M.J., 2010. Palatable cafeteria diet ameliorates anxiety and depression-like symptoms following an adverse early environment. Psychoneuroendocrinology 35 (5), 717–728.
Maniam, J., et al., 2016. A diet high in fat and sugar reverses anxiety-like behaviour induced by limited nesting in male rats: impacts on hippocampal markers. Psychoneuroendocrinology 68, 202–209.
McLaughlin, K.A., et al., 2015. Causal effects of the early caregiving environment on development of stress response systems in children. Proc. Natl. Acad. Sci. U. S. A. 112 (18), 5637–5642.
Mehta, M.A., et al., 2009. Amygdala, hippocampal and corpus callosum size following severe early institutional deprivation: the English and Romanian Adoptees study pilot. JCPP (J. Child Psychol. Psychiatry) 50 (8), 943–951.
Merz, E.C., et al., 2013. Inhibitory control and working memory in post-institutionalized children. J. Abnorm. Child Psychol. 41 (6), 879–890.
Mihalas, A., Hevner, R., 2017. Control of neuronal development by T-box genes in the brain. In: Current Topics in Developmental Biology. Elsevier, pp. 279–312.
Murray, E.A., Wise, S.P., Drevets, W.C., 2011. Localization of dysfunction in major depressive disorder: prefrontal cortex and amygdala. Biol. Psychiatr. 69 (12), e43–54.
Okonechnikov, K., Conesa, A., García-Alcalde, F., 2016. Qualimap 2: advanced multi-sample quality control for high-throughput sequencing data. Bioinformatics 32 (2), 292–294.
Pechtel, P., Pizzagalli, D.A., 2011. Effects of early life stress on cognitive and affective function: an integrated review of human literature. Psychopharmacology (Berlin) 214 (1), 55–70.
Peña, C.J., et al., 2019. Early life stress alters transcriptomic patterning across reward circuitry in male and female mice. Nat. Commun. 10 (1), 1–13.
Price, J.L., Drevets, W.C., 2012. Neural circuits underlying the pathophysiology of mood disorders. Trends Cognit. Sci. 16 (1), 61–71.
Reimand, J., et al., 2007. g: profiler—a web-based toolset for functional profiling of gene lists from large-scale experiments. Nucleic Acids Res. 35 (Suppl. l_2), W193–W200.
Reincke, S.A., Hanganu-Opatz, I.L., 2017. Early-life stress impairs recognition memory and perturbs the functional maturation of prefrontal-hippocampal-perirhinal networks. Sci. Rep. 7 (1), 1–16.
Rincel, M., et al., 2018. Maternal high-fat diet and early life stress differentially modulate spine density and dendritic morphology in the medial prefrontal cortex of juvenile and adult rats. Brain Struct. Funct. 223 (2), 883–895.
Robinson, O.J., et al., 2014. Towards a mechanistic understanding of pathological anxiety: the dorsal medial prefrontal-amygdala 'aversive amplification' circuit in unmedicated generalized and social anxiety disorders. Lancet Psychiatry 1 (4), 294–302.
Russo, S.J., Nestler, E.J., 2013. The brain reward circuitry in mood disorders. Nat. Rev. Neurosci. 14 (9), 609–625.
Shah, A.A., Treit, D., 2003. Excitotoxic lesions of the medial prefrontal cortex attenuate fear responses in the elevated-plus maze, social interaction and shock probe burying tests. Brain Res. 969 (1–2), 183–194.
Shin, L.M., et al., 2005. A functional magnetic resonance imaging study of amygdala and medial prefrontal cortex responses to overtly presented fearful faces in posttraumatic stress disorder. Arch. Gen. Psychiatr. 62 (3), 273–281.
Stamatakis, A., et al., 2016. Exposure to a mildly aversive early life experience leads to prefrontal cortex deficits in the rat. Brain Struct. Funct. 221 (8), 4141–4157.
Teissier, A., et al., 2020. Early-life stress impairs postnatal oligodendrogenesis and adult emotional behaviour through activity-dependent mechanisms. Mol. Psychiatr. 25 (6), 1159–1174.
Torres-Berrío, A., et al., 2019. Unraveling the epigenetic landscape of depression: focus on early life stress. Dialogues Clin. Neurosci. 21 (4), 341.
Tottenham, N., et al., 2010. Prolonged institutional rearing is associated with atypically large amygdala volume and difficulties in emotion regulation. Dev. Sci. 13 (1), 46–61.
Urb, M., et al., 2019. Glucocorticoid receptor stimulation resulting from early life stress affects expression of DNA methyltransferases in rat prefrontal cortex. J. Mol. Neurosci. 68 (1), 99–110.
VanTieghem, M.R., Tottenham, N., 2018. Neurobiological programming of early life stress: functional development of amygdala-prefrontal circuitry and vulnerability for stress-related psychopathology. Curr Top Behav Neurosci 38, 117–136.
Walker, C.-D., et al., 2017. Chronic early life stress induced by limited bedding and nesting (LBN) material in rodents: critical considerations of methodology, outcomes and translational potential. Stress 20 (5), 421–448.
Yang, Y., et al., 2016. Chronic stress regulates NG2(+) cell maturation and myelination in the prefrontal cortex through induction of death receptor 6. Exp. Neurol. 277, 202–214.
Yang, Y., et al., 2017. Neonatal maternal separation impairs prefrontal cortical myelination and cognitive functions in rats through activation of Wnt signaling. Cerebr. Cortex 27 (5), 2871–2884.
Zhang, S., et al., 2010. Draxin, a repulsive axon guidance protein, is involved in hippocampal development. Neurosci. Res. 66 (1), 53–61.
Zhang, Y., et al., 2014. An RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex. J. Neurosci. 34 (36), 11929–11947.