Antenatal Glucocorticoid Exposure Results in Sex-Specific and Transgenerational Changes in Prefrontal Cortex Gene Transcription that Relate to Behavioural Outcomes

Andrea Constantinof1, Vasilis G. Moisiadis1, Alisa Kostaki1, Moshe Szyf2 & Stephen G. Matthews1,3,4,5

Synthetic glucocorticoids (sGC) are administered to women at risk for preterm delivery to reduce respiratory distress syndrome in the newborn. The prefrontal cortex (PFC) is important in regulating stress responses and related behaviours and expresses high levels of glucocorticoid receptors (GR). Further, antenatal exposure to sGC results in a hyperactive phenotype in first generation (F1) juvenile male and female offspring, as well as F2 and F3 juvenile females from the paternal lineage. We hypothesized that multiple courses of antenatal sGC modify gene expression in the PFC, that these effects are sex-specific and maintained across multiple generations, and that the gene sets affected relate to modified locomotor activity. We performed RNA sequencing on PFC of F1 juvenile males and females, as well as F2 and F3 juvenile females from the paternal lineage and used regression modelling to relate gene expression and behavior. Antenatal sGC resulted in sex-specific and generation-specific changes in gene expression. Further, the expression of 4 genes (C9orf116, Calb1, Glra3, and Gpr52) explained 20–29% of the observed variability in locomotor activity. Antenatal exposure to sGC profoundly influences the developing PFC; effects are evident across multiple generations and may drive altered behavioural phenotypes.

The prefrontal cortex (PFC) is essential for top-down regulation of neuroendocrine and behavioural processes1,2. Glutamatergic efferents project from the PFC to forebrain regions that then project GABAergic efferents to the paraventricular nucleus (PVN), decreasing the hypothalamic-pituitary-adrenal (HPA) axis response to stress2,3. The PFC is also highly sensitive to environmental stimuli (e.g. stress, sleep, diet), in particular, stimuli present during fetal and/or early postnatal life4. For example, antenatal exposure to high levels of glucocorticoids (GCs) programs changes in gene expression in the PFC that persist through adulthood5. Furthermore, altered signaling of key pathways in the PFC, such as the GABAergic signaling pathway, have been implicated in many psychiatric disorders that have developmental origins, including Attention Deficit Hyperactivity Disorder (ADHD)6, post-traumatic stress disorder (PTSD), major depressive disorder (MDD), and bipolar personality disorder (BPD)7. Thus, the PFC is a critical brain region of interest to the study of the impact of fetal exposures.

Antenatal synthetic glucocorticoids (sGC) are administered to women at risk for preterm delivery to decrease the morbidity and mortality in the newborn associated with preterm birth (e.g. respiratory distress syndrome)8–10.
This life-saving treatment has also been associated with an increased risk of developing stress-related behavioural problems, including anxiety, hyperactivity, and distractibility in children born preterm and children born at term; female children are affected more than male children. sGCs exert their effects by primarily binding to glucocorticoid receptors (GR), which translocate to the nucleus and bind glucocorticoid response element (GRE) regions in the DNA to regulate gene expression. The GR is highly expressed in the developing prenatal brain, especially in the PFC. Antenatal sGC exposure alters the expression of genes related to ADHD in the prefrontal cortex of marmoset monkeys, and affects the volume of brain regions involved in regulating behaviour in human infants and children. In animal studies, we have previously demonstrated that antenatal exposure to sGC results in widespread changes in gene expression in the fetal brain. Further, the effects of sGC exposure on gene expression and behaviour transmit across multiple generations of juvenile offspring in the guinea pig in a sex-specific manner. In animal studies, we have previously demonstrated that antenatal exposure to sGC results in widespread changes in gene expression in the fetal brain. Further, the effects of sGC exposure on gene expression and behaviour transmit across multiple generations of juvenile offspring in the guinea pig in a sex-specific manner. In animal studies, we have previously demonstrated that antenatal exposure to sGC results in widespread changes in gene expression in the fetal brain. Further, the effects of sGC exposure on gene expression and behaviour transmit across multiple generations of juvenile offspring in the guinea pig in a sex-specific manner.
genes down-regulated. GSEA revealed 56 enriched gene sets (Supplementary Table 1; NES > 1.6, FDR < 0.25); 53 were positively enriched and 3 negatively enriched; NES > 1.6, FDR < 0.25). In F3 offspring, 438 genes were significantly differentially expressed in the sGC group compared to controls (Fig. 1C; P < 0.001, FDR < 0.05), 258 genes were significantly up-regulated and 180 genes down-regulated; NES > 1.6, FDR < 0.25. GSEA identified 162 enriched gene sets, with 116 positively enriched and 46 negatively enriched (Supplementary Table 1; NES > 1.6, FDR < 0.25). There were 22 genes that significantly differentially expressed in all three generations of female offspring (Fig. 2, FDR < 0.05).

**Gene Expression: Males.** In the F1 juvenile males, a total of 996 genes were significantly differentially expressed in sGC offspring relative to control (Fig. 1D; FDR < 0.05). Of the differentially expressed genes, 354 were significantly down-regulated and 642 genes were significantly up-regulated. GSEA identified 157 gene sets that were significantly enriched in F1 sGC male offspring compared to controls. 48 gene sets were negatively enriched in the F1 sGC male offspring, while 109 pathways were positively enriched (Supplementary Table 1; NES > 1.6, FDR < 0.25). There were 22 genes that significantly differentially expressed in all three generations of female offspring (Fig. 2, FDR < 0.05).

**Gene Expression: Female vs. Male Comparisons.** There were 215 genes that were significantly differentially expressed in the PFC from F1 sGC female and male offspring (FDR < 0.05; Supplementary Table 2; Fig. 3A). There were 22 genes that were down-regulated in both male and female offspring whose mothers had been exposed to sGC (Supplementary Table 2) and were shown to be significantly enriched for the locomotor behavior pathway by ConsensusPathDB (p < 0.001, FDR < 0.05). The expression of the remaining 193 genes was divergent in males and females (i.e. up in males and down in females, or vice-versa; Fig. 3B). GSEA showed 51 gene sets were enriched in both sGC female and male offspring, however the enrichment occurred in opposite directions in each sex (i.e. increased in males and down in females, or vice-versa; NES > 1.6, FDR < 0.25; Supplementary Table 3). Since the sGC offspring in all four groups of sGC animals (F1-F3 Females and F1 males) displayed increased open-field activity, we investigated common genes that were differentially expressed in all four groups. The hypothesis was that despite sex-specific changes in gene expression, there may be genes common to all groups that are associated with the observed open-field activity in these animals. Ten genes: Arpp21, Atppap11, C9orf116, Calb1, Ghr3, Gpr52, Krt80, Pdyn, Sowaha, Vstm2l, were differentially expressed in all four groups of sGC animals (FDR < 0.05; F1-F3 Females and F1 males; Table 1).

**Regression Results.** Recursive feature selection was used to rank the 10 genes that were differentially expressed in all four groups based on their contribution to the variation in open-field activity (Table 2). Multivariate linear regression was used to model the relationship between gene expression and behavior, with the

![Figure 2. (A) Venn diagram illustrating the number of genes that were significantly differentially expressed (P < 0.001, FDR < 0.05) in the PFC from F1-F3 sGC females and the number of genes that overlap between generations. (B) Expression changes of the 22 genes that were differentially expressed (P < 0.001, FDR < 0.05) in all three generations of female offspring. Values indicate the fold-change in gene expression in sGC animals relative to control, color further indicates the direction of change (green: significantly down-regulated, red: significantly upregulated).](image-url)
Figure 3. (A) Venn diagram illustrating the number of genes that were significantly differentially expressed in the PFC from F₁ female and F₁ male sGC offspring and the number of genes that overlap between generations. (B) Heatmap of the 215 genes that were differentially expressed in F₁ female and F₁ male sGC offspring (P < 0.001, FDR < 0.05). Each row represents one gene, each column represents one animal. Green represents low expression and red represents high expression.

Table 1. Expression changes of the 10 genes that are differentially expressed in all four groups of sGC offspring. Values indicate the fold-change in gene expression in sGC animals relative to control. Positive numbers indicate significantly upregulated expression, negative numbers indicate significantly down-regulated expression.

| Gene   | F₁ Males | F₁ Females | F₂ Females | F₃ Females |
|--------|----------|------------|------------|------------|
| Arpp21 | −1.27    | −1.78      | −1.41      | −1.90      |
| Atp6ap1l | −1.18    | 1.98       | −1.29      | 1.40       |
| C9orf116 | −1.47    | −2.45      | −1.73      | −2.08      |
| Calb1  | −1.96    | −3.05      | −2.10      | −3.18      |
| Glra3  | −3.73    | −2.62      | −2.75      | −4.30      |
| Gpr52  | −2.28    | −2.50      | −2.16      | −4.97      |
| Krt80  | −2.78    | −4.46      | −2.37      | −3.84      |
| Pdyn   | −3.08    | −3.06      | −3.39      | −2.62      |
| Sowaha | −1.51    | −2.64      | −1.58      | −2.22      |
| Vstm2l | −1.62    | −1.59      | −1.47      | −1.88      |

Table 2. Gene ranking after recursive feature selection.

| Gene    | Rank |
|---------|------|
| C9orf116 | 1    |
| Glra3   | 2    |
| Gpr52   | 3    |
| Calb1   | 4    |
| Krt80   | 5    |
| Sowaha  | 6    |
| Pdyn    | 7    |
| Atp6ap1l | 8    |
| Arpp21  | 9    |
| Vstm2l  | 10   |

best model being made with the inclusion of the top four genes C9orf116, Calb1, Glra3, and Gpr52 from recursive feature selection (Fig. 4; adjusted R² = 0.29, P = 0.006). The prediction model was validated after leave-one-out cross-validation (Supplementary Fig. 1; adjusted R² = 0.20, P = 0.004).
Discussion

Antenatal exposure to sGC resulted in changes to gene expression in the PFC that persist across three generations of juvenile female offspring derived through the paternal lineage. We previously demonstrated that F1-F3 female offspring and F1 male offspring display a hyperactive phenotype in the open-field test. Here, we observed striking sex-specific effects of sGC on gene transcription in the PFC, with a small overlap (~10%) in the number of genes that were affected by sGC in F1 females and males. While 193 genes were differentially expressed in opposite directions, there were 22 genes that were down regulated in both male and female sGC F1 offspring, and these genes were enriched for locomotor activity. Furthermore, we identified four differentially expressed genes in F1-F3 female offspring and F1 male offspring that were associated with 20–29% of the open-field activity variability, thereby providing insight into changes in gene expression following sGC that may mediate behavioural outcomes in both male and female offspring.

Transgenerational Effects of Antenatal sGC and GABAergic Expression Pathways. In F1 offspring, the expression of GABAergic signaling genes (Gabra3a, Gad2) were significantly altered in the PFC of animals exposed to antenatal sGC. Gad2, that encodes glutamic acid decarboxylase was significantly down-regulated in the sGC females, while Gabra3a, the primary GABA receptor in PFC neurons, was significantly up-regulated. These changes may indicate that F1 female offspring of sGC-treated mothers had decreased GABA neurotransmitter levels, which has previously been shown to result in increased HPA function and a hyperactive phenotype. Furthermore, the expression of GABAergic signaling genes, Gabra2 and Gabra3a were significantly down-regulated in F1 sGC offspring, while Gabra1 and Gabra3 were significantly upregulated, with Gad2 significantly down regulated in F1 sGC animals. These data indicate that altered gene expression related to GABAergic signaling persists over multiple generations. Altered GABAergic signaling in the PFC has been previously observed in patients with schizophrenia, bipolar disorder, and major depressive disorder, and early life exposure to sGC has been linked to development of psychiatric disease. Therefore, the changes in expression for GABAergic genes that we observe following exposure to sGC may be associated with increased risk of psychiatric disease later in life.

Sex-Specific Effects of Antenatal sGC on PFC Gene Expression: Open-Field Activity. Prenatal exposure to sGC resulted in substantial changes in gene expression in the PFC that extended, at least, up to 50 days after exposure in F1 male and female offspring. Consistent with previous literature, we observed sex- and generation-specific programming following antenatal sGC exposure. All 51 commonly enriched gene sets were affected in the opposite direction in male and female offspring. The gene sets most affected included extracellular ligand-gated ion channel activity (critical for intercellular communication) and synaptic signaling (synapse formation). Both pathways were up-regulated in females, and down-regulated in males. These pathways play a pivotal role in information processing allowing appropriate behavioural responses and adaptation. Since these pathways were enriched in opposite directions in males and females, it is possible that simply perturbing these pathways is sufficient to produce a hyperactive phenotype. Conversely, enrichment of these pathways may not play a significant role in the observed hyperactive phenotype, and further detailed investigation is required.

Greater insight regarding the relationship between gene expression and behaviour may come from the genes that were significantly differentially expressed in both male and female sGC offspring. Of the 215 genes that were differentially expressed in both male and female offspring, 193 were expressed in opposite directions, but there were 22 genes that were significantly down-regulated in both sGC male and female F1 offspring, and these genes...
were enriched for the locomotor behaviour pathway. These findings suggest that despite the major sex-specific differences in gene expression, the hyperactive phenotype observed in both males and females may be mediated by the same transcriptional pathways in both sexes.

**Transgenerational Effects of Antenatal sGC: Molecular and Behavioural Correlations.** Since all three generations of sGC females and F1 males displayed increased open field activity, we investigated changes in gene expression that occurred in all four groups of sGC offspring to identify genes related to the behavioural phenotype. There were 10 genes (Arpp21, At1pap1, C9orf116, Calb1, Gpra3, Gpr52, Krt80, Pdyn, Sowaha, Vstm2) significantly differentially expressed in all three generations of female offspring and in the F1 males. It is important to note that the expression of these 10 genes was not altered in the PVN of the same female offspring following antenatal sGC exposure, indicating region-specific effects. Feature selection analysis and multivariate linear regression analysis suggest that the expression of four of these genes, Chromosome 9 Open Reading Frame 116 (C9orf116), Calbindin 1 (Calb1), Glycine Receptor Alpha 3 (Glr3a), and G Protein-Coupled Receptor 52 (Gpr52), are involved in the hyperactive behavioural phenotype observed in the sGC-exposed offspring lineage. The expression of these genes was significantly decreased in all four groups of sGC animals. While these genes have not been previously studied in the context of antenatal sGC exposure and locomotor activity, each gene plays an essential role in processes that are integral to governing locomotor behaviour.

**C9orf116** expression is directly regulated by p53, and C9orf116 knockdown down-regulates proapoptotic genes, implicating a role in apoptosis. Reduced expression of genes involved in apoptosis has previously been observed in isolation-reared rats that displayed a hyperactive phenotype in the open-field, and may be related to changes in apoptotic levels that alter neural plasticity in the PFC.

**Calb1** is a high-affinity calcium buffer/sensor in pyramidal, nonpyramidal, and GABAergic interneurons in the PFC. Calb1 has a protective effect against neuronal injury from excess Ca2+ exposure. Calb1 is regulated by estrogen and androgens, creating sex-specific differences in its expression. Antenatal sGC exposure decreases Calb1 expression in the basolateral amygdala and Calb1 expression is decreased in rats weaned in isolation, resulting in decreased exploratory behaviour. Calb1 knock-out animals display decreased expression of GABAergic signaling genes (previously linked to hyperactive phenotype), which is consistent with the changes observed in the sGC offspring in the present study. Therefore, Calb1 expression has been shown to be affected by antenatal sGC and altered expression has been shown to influence open-field activity. The observed decrease in Calb1 expression in the sGC offspring may influence open-field activity through GABAergic interactions.

**Gpr52** is an orphan g-protein coupled receptor that is expressed exclusively in the brain. Gpr52 knock-out has anxiolytic effects on behaviour in mice. In humans, GPR52 expression profiles overlap with the distribution of D1 dopamine receptors in the PFC, and it is thought that the expression of Gpr52 influences locomotor activity through activation of the dopamine receptor D1 (DRD1) and N-methyl-D-aspartate (NMDA) receptors in the PFC through intracellular CAMP accumulation. Of note, Drd1 expression is significantly down-regulated in F1 sGC females, and significantly upregulated in F2 sGC females, while expression of Grin2a, which encodes for the NMDA receptor, is significantly upregulated in F2 sGC females, which may present a plausible mechanism by which the decreased Gpr52 expression observed in the sGC offspring influences open-field activity.

Glycine receptors, such as Glr3a play a fundamental role in mediating inhibitory neurotransmission throughout the central nervous system. Glycine receptor knock-out animals show increased locomotor activity in the open-field when stimulated with low levels of ethanol. This may occur due to neuronal disinhibition from reduced effects of ethanol on glycine receptors. The decreased Glr3a expression in the sGC animals may increase neuronal disinhibition, and play a role in the increased open-field activity observed in the sGC-exposed offspring lineage.

The reduced expression of these four genes, selected from recursive feature selection analyses, explained between 20–29% of the variability in hyperactive behaviour observed in F1 males and F1-F3 juvenile female offspring. While altered expression of these genes has previously been shown to influence locomotor activity, future experiments are required to investigate the specific mechanisms by which decreased expression of Calb1, Glr3a, Gpr52, C9orf116 in the PFC alter open-field activity in the context of antenatal sGC exposure. Though changes in gene expression in the PFC can provide some insight into the sources of variability contributing to increased open-field activity, 70–80% of the variability remains to be explained. The PFC has glutamatergic efferents that directly connect to the ventral tegmental area (VTA) and the nucleus accumbens (Nac), which have been connected to locomotor activity. It is possible that dysregulated gene expression in the PFC has downstream effects in other brain regions that contribute to the hyperactive phenotype observed in the sGC offspring and merit further investigation. It is also possible that gene expression changes in the PFC and behaviours are independent and may be a result of parallel downstream effects of sGC, though given the pivotal role that the PFC plays in behaviour, this would appear unlikely.

These findings demonstrate paternal transmission of the effects of antenatal sGC over three generations of female offspring, yet the mechanism of transmission has yet to be elucidated. We have shown that antenatal sGC exposure results in a complex pattern of effects that are dynamic and dependent on sex, age, generation, brain region, and parental line of transmission, which is consistent with other instances of transgenerational transmission. Unique to the present study is the identification of select genes that are consistently altered across all four groups of sGC offspring and relate to a hyperactive phenotype. These findings may indicate that PFC signaling plays a critical role in propagating the effects of antenatal sGC.

**Conclusion** We have demonstrated transgenerational changes in gene expression that relate to the behavioural phenotypes observed in the juvenile offspring. Antenatal exposure to sGC resulted in a pattern of gene expression in the PFC consistent with reduced GABAergic signaling in F1-F3 offspring. As disruption of GABAergic signaling is common in major psychiatric diseases, and as sGC expression is associated with increased risk for developing
psychiatric disease25, this pattern of gene expression may provide a mechanism by which antenatal sGC exposure contributes to psychiatric vulnerability. Despite observing major sex- and generation-specific differences in the effects of sGC on gene expression, we identified four genes that may contribute to 20–29% of the variability in locomotor activity in F1 sGC males and all three generations of sGC female offspring. These findings demonstrate that multiple courses of antenatal sGC result in permanent changes in gene expression that likely alter phenotype over three generations. Follow-up studies in human cohorts are imperative to ascertain the long-term effects of sGC on neural development.

Materials and Methods

Animals. Pregnant guinea pigs received 3 courses of the sGC betamethasone (sGC; 1 mg/kg) or saline control in late gestation, as previously described18. The dose of sGC used is comparable to that administered to pregnant women at risk of preterm delivery (~0.25 mg/kg) as the glucocorticoid receptor (GR) in guinea pigs has a 4-fold lower affinity for sGC42. First (F1) and second (F2) generation male offspring were mated with non-experimental females to generate F3 and F4 offspring, as previously described18. Total locomotor activity in the open-field test (open-field activity; OFA) was measured in female and male offspring on postnatal day 19, and brains were collected at day 40, as previously reported18. The locomotor activity in the open-field, of the animals used for molecular analysis in the present study, was presented previously25. The right frontal cortex from the F1 males and F1-F3 paternal line females were cryosectioned at −20 °C. 1.0 mm diameter punches (Harvard Apparatus Inc., Holliston, MA, USA) of the mPFC, cingulate cortex area 1 and infralimbic cortex were taken from F1 (Control; n = 4, sGC; n = 4), F2 (Control; n = 4, sGC; n = 4), and F3 (Control; n = 4, sGC; n = 4) females and F1 males (Control; n = 5, sGC; n = 6) as previously reported48. Only one animal of each sex from each litter was used in the molecular analysis of female offspring. Animals for RNA-seq analysis were selected based on the availability of sufficient high-quality RNA. All protocols were approved by the Animal Care Committee at the University of Toronto in accordance with the Canadian Council on Animal Care.

RNA Sequencing. RNA was extracted from punches using the AllPrep Universal Kit (Qiagen, Ontario, Canada) and RNA quality was determined by Bioanalyzer (RNA 6000 Pico LabChip, Applied Biosystems, Ontario, Canada); all RNA samples RIN ≥ 7. mRNA library preparation was performed using Illumina TruSeq V2 mRNA enrichment using standard protocols. High-throughput sequencing were performed on an Illumina HiSeq2500 sequencing system using standard run, following the protocol recommended by Illumina for sequencing mRNA samples. Sequencing was undertaken for each biological replicate at 1 × 51 bp (Donnelly Centre for Cellular and Biomolecular Research; Toronto, Canada). RNA-seq results were analyzed, as previously described18. Briefly, differential gene expression was assessed using EdgeR (version 3.12.1)43,44 general linear model likelihood determination was calculated. The model was validated using leave-one-out cross validation.

Behavioural and Molecular Correlations. To identify genes that are associated with locomotor activity, recursive feature selection48 was performed on the normalized gene expression counts for the genes that were significantly differentially expressed in F1 sGC males, and in all 3 generations of sGC female animals. First, the expression of all the genes were fitted in a linear regression to predict open field activity. The coefficients of each gene were used to rank the genes from highest contribution to open field activity to lowest. The gene with the lowest contribution to open-field activity was removed, and the remaining genes were fitted in a new linear regression to predict open-field activity. This process was repeated until all the genes were ranked in order of contribution (or importance) to open-field activity48. The top four feature selected genes (C9orf116, Calb1, Gfra3, and Gpr52) were input into a multiple regression to predict open-field activity, and the coefficient of determination was calculated. The model was validated using leave-one-out cross validation.

Data Availability

All sequencing data can be found under GEO submission ID: GSE107415. Computer code available upon request.

References

1. Arnsten, A. F. T. Stress signalling pathways that impair prefrontal cortex structure and function. Nature Reviews Neuroscience 10, 410–422 (2009).
2. McKeon, J. M., Myers, B. & Herman, J. P. The medial prefrontal cortex: coordinator of autonomic, neuroendocrine, and behavioral responses to stress. Journal of Neuroendocrinology 27, 446–456 (2015).
3. Radley, J. J., Gosselink, K. L. & Sawchenko, P. E. A discrete GABAergic relay mediates medial prefrontal cortical inhibition of the neuroendocrine stress response. Journal of Neuroscience 29, 7330–7340 (2009).
4. Kolb, B. et al. Experience and the developing prefrontal cortex. Proceedings of the National Academy of Sciences 109, 17186–17193 (2012).
5. Diaz Heijtz, R., Fuchs, E., Feldon, J., Pryce, C. R. & Forsberg, H. Effects of antenatal dexamethasone treatment on glucocorticoid receptor and calcyton gene expression in the prefrontal cortex of neonatal and adult common marmoset monkeys. Behavioral and Brain Functions 6, 18 (2010).

6. Cheng, J., Liu, A., Shi, M. Y. & Yan, Z. Disrupted glutamatergic transmission in prefrontal cortex contributes to behavioral abnormality in an animal model of ADHD. Neuropsychopharmacology 42, 2096–2104 (2017).

7. Sala, M. et al. Stress and hippocampal abnormalities in psychiatric disorders. European Neuropsychopharmacology 14, 393–405 (2004).

8. Liggins, G. C. & Howie, R. N. A controlled trial of antepartum glucocorticoid treatment for prevention of the respiratory distress syndrome in premature infants. Pediatrics 50, 515–525 (1972).

9. Antenatal corticosteroids revisited: repeat courses - National Institutes of Health Consensus Development Conference Statement, August 17–18, 2000. Obstetrics and Gynecology 98, 144–150 (2001).

10. Moniadiis, V. C. & Matthews, S. G. Glucocorticoids and fetal programming part 1: Outcomes. Nature Reviews Endocrinology 10, 391–402 (2014).

11. Cartier, J., Zeng, Y. & Drake, A. J. Glucocorticoids and the prenatal programming of neurodevelopmental disorders. Current Opinion in Behavioral Sciences 7, 1–7 (2016).

12. Kemp, M. W., Newnham, J. P., Challis, J. G., Joe, A. H. & Stock, S. J. The clinical use of corticosteroids in pregnancy. Human Reproduction Update 22, 240–259 (2016).

13. Truss, M. & Beato, M. Steroid hormone receptors: interaction with deoxyribonucleic acid and transcription factors. Endocrine Reviews 14, 459–479 (1993).

14. Diorio, D., Viau, V. & Meaney, M. J. The role of the medial prefrontal cortex (cingulate gyrus) in the regulation of hypothalamic-pituitary-adrenal responses to stress. The Journal of Neuroscience 13, 3839–3847 (1993).

15. Tsujieling, D. et al. Effects of Antenatal Glucocorticoid Therapy on Hippocampal Histology of Preterm Infants. PloS One 7, e33369, https://doi.org/10.1371/journal.pone.0033369 (2012).

16. Davis, E. P., Sandman, C. A., Wing, D. A. & Head, K. Fetal glucocorticoid exposure is associated with preadolescent brain development. Biological Psychiatry 74, 647–653 (2015).

17. Crudo, A. et al. Effects of antenatal synthetic glucocorticoid on glucocorticoid receptor binding, DNA methylation, and genome-wide mRNA levels in the fetal male hippocampus. Endocrinology 154, 4170–4181 (2013).

18. Moisissiadis, V. G., Constantinoif, A., Kostaki, A., Sayf, M. & Matthews, S. G. Prenatal glucocorticoid exposure modifies endocrine function and behaviour for 3 generations following maternal and paternal transmission. Scientific reports 7, 11814 (2017).

19. Dobbing, I. & Sands, J. Comparative aspects of the brain growth spurt. Early Human Development 3, 79–83 (1979).

20. Leiser, R. & Kaufmann, P. Placental structure: in a comparative aspect. The Journal of Physiology 201, 132–134 (1964).

21. Brambilla, P., Perez, J., Barale, F., Schettini, G. & Soares, J. C. GABAergic dysfunction in mood disorders. Mol. Psychiatry 8, 721–737 (2003).

22. Shen, Q. et al. Aminobutyric acid-type A receptor deficits cause hypothalamic-pituitary-adrenal axis hyperactivity and antidepressant drug sensitivity reminiscent of melancholic depression. Biological Psychiatry 68, 512–520 (2010).

23. Asinof, S. K. & Paine, T. A. Inhibition of GABA synthesis in the prefrontal cortex increases locomotor activity but does not affect attention in the 5-choice serial reaction time task. Neuropharmacology 65, 39–47 (2013).

24. Ghosal, S., Hare, B. D. & Duman, R. S. Prefrontal cortex GABAergic deficits and circuit dysfunction in the pathophysiology and treatment of chronic stress and depression. Current Opinion in Behavioral Sciences 14, 1–8 (2017).

25. Khalife, N. et al. Prenatal glucocorticoid treatment and later mental health in children and adolescents. PloS One 8, e81394, https://doi.org/10.1371/journal.pone.0081394 (2013).

26. Igpal, M., Moisissiadis, V. G., Kostaki, A. & Matthews, S. G. Transgenerational effects of prenatal synthetic glucocorticoids on hypothalamic-pituitary-adrenal function. Endocrinology 153, 3295–3307 (2012).

27. Li, S., Wong, A. H. C. & Liu, F. Ligand-gated ion channel interacting proteins and their role in neuroprotection. Frontiers in Cellular Neuroscience 8, https://doi.org/10.3389/fncel.2014.00125 (2014).

28. Töth, K. Synaptic signalling and plasticity: emerging new players. The Journal of Physiology 594, 5439–5440 (2016).

29. Sung, Y. H. et al. Pircel, a novel p53 target gene contributing to the ultraviolet-induced DNA damage response. Cancer Research 70, 10454–10463 (2010).

30. Levine, J. B. et al. Isolation rearing and hyperlocomotion are associated with reduced immediate early gene expression levels in the medial prefrontal cortex. Neuroscience 145, 42–55 (2007).

31. Harris, E. P., Abel, J. M., Tejada, L. D. & Rissman, E. F. Calbindin knockout alters sex-specific regulation of behavior and gene expression in amygdala and prefrontal cortex. Endocrinology 157, 1967–1979 (2016).

32. Mattson, M. P., Rychlik, B., Chiu, C. & Christakos, S. Evidence for calcium-reducing and excitoprotective roles for the calcium-binding protein calbindin-D28k in cultured hippocampal neurons. Neuron 6, 41–51 (1991).

33. Zuloaga, D. G., Carbone, D. L. & Handa, R. J. Prenatal dexamethasone selectively decreases calretinin expression in the adult female lateral amygdala. Neuroscience Letters 521, 109–114 (2012).

34. Pascual, R., Zamora-León, P., Catalan-Ahumada, M. & Valero-Cabre, A. Early social isolation decreases the expression of calbindin-D28k and dendritic branching in the medial prefrontal cortex of the rat. The International Journal of Neuroscience 117, 465–476 (2007).

35. Komatsu, H. et al. Altered sedative effects of ethanol in mice with candidate GPR52 for psychiatric disorders. PloS One 9, e81094, https://doi.org/10.1371/journal.pone.0081094 (2014).

36. Komatsu, H. Novel Therapeutic GPCRs for Psychiatric Disorders. International Journal of Molecular Sciences 16, 14109–14121 (2015).

37. Setho, M. et al. Discovery of the first potent and orally available agonist of the orphan G-protein-coupled receptor 52. Journal of Medicinal Chemistry 57, 5226–5237 (2014).

38. Briggs, C. A. & Gopalakrishnan, M. In Comprehensive Medicinal Chemistry II (ed. David J. Triggle) 877–918 (Elsevier, 2007).

39. Aguiar, L. G. et al. Altered sedative effects of ethanol in mice with r1 glycine receptor subunits that are insensitive to GABA modulation. Neuropsychopharmacology 39, 2358–2358 (2014).

40. Takahata, R. & Moghaddam, B. Activation of glutamate neurotransmission in the prefrontal cortex sustains the motoric and dopaminergic effects of phencyclidine. Neuropsychopharmacology 28, 1117–1124 (2003).

41. Bale, T. L. Epigenetic and transgenomic reprogramming of brain development. Nature Reviews Neuroscience 16, 332–344 (2015).

42. Kiehlty, M. C., Curtis, A. J., Chu, S. & Fuller, P. J. Structural determinants of cortisol resistance in the guinea pig glucocorticoid receptor. Endocrinology 139, 2479–2485 (1998).

43. Robinson, M. D., McCarthy, D. J. & Smyth, G. K. edgΔ: A Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26, 139–140 (2010).

44. Zhou, X., Lindsay, H. & Robinson, M. D. Robustly detecting differential expression in RNA sequencing data using observation weights. Nucleic Acids Research 42, e91, https://doi.org/10.1093/nar/gku031 (2014).

45. Subramanian, A. et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proceedings of the National Academy of Sciences 102, 15545–15550 (2005).
Acknowledgements

We are grateful to Dr. B. Cox for providing advice on bioinformatic analysis. This work was supported by grants from the Canadian Institutes for Health Research (CIHR; MOP-126166; FDN-148368). AC received a scholarship from Brain Canada and Kids Brain Health Network. VGM received funding from the Queen Elizabeth II Graduate Scholarships in Science and Technology and the Ontario Graduate Scholarship. The funding source had no involvement in the final design of the study, in the collection, analysis, and interpretation of data, in the writing of the manuscript, or in the decision to submit the article for publication.

Author Contributions

Each of the authors has contributed to the production of the manuscript, have consented to having their names on the manuscript and approve the final version of the manuscript. Individual contributions are as follows: A.C. contributed to the conception and design of research, acquisition of the data, analysis and interpretation of the data, drafting the manuscript, and revising/editing the manuscript. V.G.M. contributed to the acquisition of the data, analysis and interpretation of the data, drafting the manuscript, and revising/editing the manuscript. A.K. contributed to the conception and design of research, acquisition of the data, and revising/editing the manuscript. M.S. contributed to the interpretation of data, drafting the manuscript, and revising/editing the manuscript. S.G.M. contributed to the conception and design of research, interpretation of the data, drafting the manuscript, and revising/editing the manuscript.

Additional Information

Supplementary information accompanies this paper at https://doi.org/10.1038/s41598-018-37088-3.

Competing Interests: The authors declare no competing interests.

Publisher’s note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2019