Transcriptional signatures of steroid hormones in the striatal neurons and astrocytes

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Abstract

Background: The mechanisms of steroids actions in the brain mainly involve the binding and nuclear translocation of specific cytoplasmic receptors. These receptors can act as transcription factors and regulate gene expression. However, steroid-dependent transcriptional regulation in different types of neural cells is not yet fully understood. The aim of this study was to evaluate and compare transcriptional alterations induced by various steroid receptor agonists in primary cultures of astrocytes and neurons from mouse brain.

Results: We utilized whole-genome microarrays (Illumina Mouse WG-6) and quantitative PCR analyses to measure mRNA abundance levels. To stimulate gene expression we treated neuronal and astroglial cultures with dexamethasone (100 nM), aldosterone (200 nM), progesterone (200 nM), 5α-dihydrotestosterone (200 nM) and β-Estradiol (200 nM) for 4 h. Neurons were found to exhibit higher levels of expression of mineralocorticoid receptor, progesterone receptor and estrogen receptor 2 than astrocytes. However, higher mRNA level of glucocorticoid receptor mRNA was observed in astrocytes. We identified 956 genes regulated by steroids. In astrocytes we found 381 genes altered by dexamethasone and 19 altered by aldosterone. Functional classification of the regulated genes indicated their putative involvement in multiple aspects of cell metabolism (up-regulated Slc2a1, Pdk4 and Slc45a3) and the inflammatory response (down-regulated Ccl3, Il1b and Tnf). Progesterone, dihydrotestosterone and estradiol did not change gene expression in astrocytes. We found no significant changes in gene expression in neurons.

Conclusions: The obtained results indicate that glial cells might be the primary targets of transcriptional action of steroids in the central nervous system. Substantial changes in gene expression driven by the glucocorticoid receptor imply an important role for the hypothalamic–pituitary–adrenal axis in the hormone-dependent regulation of brain physiology. This is an in vitro study. Hence, the model may not accurately reflect all the effects of steroids on gene expression in neurons in vivo.

Background

The crucial components of the neuroendocrine system are steroid hormones. They affect the central nervous system by regulating allostasis and by coordinating the responses to external and internal stimuli [1]. The most prominent example of steroid impact on the brain is the release of glucocorticoids after stressful stimulus [2]. Elevated levels of these adrenal steroids may subsequently affect sleep [3], cognition [4], memory [5] or mood [6]. Moreover, our previous research revealed that glucocorticoids mediate the effects on gene expression in the striatum induced by multiple psychoactive drugs, including antidepressants (fluoxetine, mianserin), antipsychotics (risperidone, clozapine) and drugs of abuse (heroin, ethanol) [7, 8]. This is probably caused by both direct and indirect actions of psychoactive drugs on the hypothalamus and later on the pituitary and adrenal glands [8]. Furthermore, the association between dysregulation of the hypothalamic–pituitary–adrenal (HPA) axis and major depression is among the most consistent biological findings in psychiatry [9]. The other steroids including
mineralocorticoids, androgens, estrogens and progestogens also influence the central nervous system [10, 11].

Steroids bind to nuclear steroid receptors, which then bind to their response elements on DNA, activating gene transcription and causing changes in gene expression. The alterations in transcript abundance mediated by these receptors are diverse and vary among cell types. It has been shown that the cell-type-specific profile of glucocorticoid receptor occupancy is predetermined by differences in the chromatin accessibility pattern [12]. Moreover, various cell populations differ in levels of steroid receptor genes expression. The most remarkable difference between various cell types in the central nervous system is high expression of progesterone receptor (Pgr) in neurons in contrast to glial and epithelial cells [13]. This cellular specificity contrasts with the lower specificity of steroid receptor ligands and DNA response elements. For example, corticosterone—an adrenal steroid—binds to both mineralocorticoid and glucocorticoid receptors [14]. Furthermore, the differences in androgen and glucocorticoid receptor DNA binding properties are quantitative rather than qualitative [15].

The crucial aspect of steroids action on the central nervous system is how these hormones affect various cellular populations involved in control of behaviour. It is particularly interesting in the striatum, a brain region that is responsible for steroid-related neuropsychiatric phenotypes including mood control [16] and reward processing [17]. To date, there have been few studies comparing various neural cell-type-specific responses to steroids. Slezak et al. [18] compared the responses of astrocytes and neurons to dexamethasone. Jenkins et al. [19] compared the responses of oligodendrocytes, astrocytes and microglia to the same steroid. The transcriptional responses of these cell types to dexamethasone were diverse. Therefore, we aimed to infer the role of various steroids in the function of astrocytes and neurons, based on gene expression changes caused by these substances. Here, we present gene expression profiling of primary neuronal and astroglial cultures treated with various steroid receptor agonists: dexamethasone, aldosterone, progesterone, estradiol and dihydrotestosterone.

Methods

Animals

The animals used in the study were from the C57BL/6 J inbred strain colony that is maintained at the animal facility of the Institute of Pharmacology of the Polish Academy of Sciences in Krakow, Poland. The mice were housed 6–10 per cage, under a 12-h dark/light cycle, with free access to food and water. The mice had not been subjected to any prior testing. The experimental protocols used in the study were approved by the local Bioethics Commission at the Institute of Pharmacology, Polish Academy of Sciences (Krakow, Poland).

Primary cultures of striatal neurons and astrocytes

The ex vivo experiments were conducted using mouse striatal neurons and astrocytes from primary cultures. Neuronal tissues were dissected from mouse embryos at 17–18 days of gestation. The tissues were cut into small pieces and digested with trypsin (0.1% for 15 min at RT). The cells were suspended in Neurobasal medium (Gibco) and plated onto polyornithine multiwell plates (TPP). After 2 days, the culture medium was replaced with Neurobasal medium supplemented with B27 (50 µl/100 ml). This procedure typically yields cultures that contain >90% neurons (Fig. 1). Primary astrocytes were isolated from P5 to P7 mice. The brains were collected, dissected and digested using the Neural Tissue Dissociation Kit (T) (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany) according to the manufacturer’s protocol. Astrocytes from single-cell suspensions were isolated using the Anti-GLAST (ACSA-1) MicroBead Kit (Miltenyi Biotec), separated on MACS MS Columns using the MACS magnetic separator, seeded on 24-well plates and grown in high-glucose DMEM supplemented with 10% FBS and antibiotics for 7 days. All cell cultures were maintained at 37 °C in a humidified atmosphere containing 5% CO₂ for 7 days prior to experiments. Both astrocytes and neurons were cultured in Neurobasal medium with no supplementation for the final 24 h.

Steroid ligands ex vivo treatments

Both neurons and astrocytes were exposed to dexamethasone (100 nM), corticosterone (10, 50 and 200 nM) aldosterone (200 nM), progesterone (200 nM), 5α-dihydrotestosterone (200 nM), or β-Estradiol (200 nM) for 4 h and 8 h (corticosterone only). The doses were selected based on previous studies [20–22]. To obtain stock solutions 1 mg of each compound was dissolved in 100% ethanol and then diluted 50 times in neurobasal medium without supplements. As a result of dissolution we obtained stock solutions of dexamethasone (51 µM), corticosterone (57 µM), β-estradiol (73 µM), progesterone (64 µM), aldosterone (55 µM) and testosterone (69 µM). The highest final solution of ethanol in culture medium was below 0.004% and this concentration was used as the vehicle negative control. The 4 h time point was selected based on previous in vitro studies using dexamethasone [18] and in vivo studies using progesterone and glucocorticoid receptor antagonist RU486 [8].
RNA isolation
RNA was isolated following the manufacturer’s protocol and was further purified using the RNeasy Mini Kit (Qiagen Inc.). Total RNA concentration was measured using a NanoDrop ND-1000 (NanoDrop Technologies Inc., Montchanin, DE, USA). RNA quality was determined using an Agilent 2100 Bioanalyzer (RIN > 8) (Agilent, Palo Alto, CA, USA).

Quantitative PCR
Reverse transcription was performed using Omniscript Reverse Transcriptase (Qiagen Inc.). TaqMan® probes (Life Technologies) were used for the qPCR assays, which were run on the CFX96 Real-Time system (Bio-Rad). Each template was generated from an individual sample, and the amplification efficiency for each assay was determined by running a standard dilution curve. Expression of the hypoxanthine–guanine phosphoribosyltransferase 1 (Hprt1) transcript, which showed stable levels following the treatment was quantified to control for variation in cDNA amounts. The abundance of RNA was calculated as $2^{-\Delta \text{Ct}}$ (threshold cycle). Data were analyzed using Student’s t test.

Gene expression profiling
First, 200 ng high-quality total RNA was used to generate cDNA and cRNA with the Illumina TotalPrep RNA Amplification Kit (Illumina Inc., San Diego, CA, USA). The procedure consisted of reverse transcription with an oligo(dT) primer bearing a T7 promoter using an Array-Script. The obtained cDNA became a template for in vitro transcription using T7 RNA polymerase and

Fig. 1 Primary cell cultures of neurons and astrocytes. The purity was estimated by immunostaining for specific protein markers. (a) Neuronal cultures were immunostained on glass slides against MAP2 protein (red), GFAP astrocyte specific protein (green) and DAPI (blue). The staining confirmed at least 90% purity of neuronal cultures from the mouse striatum. (b) Primary astrocytes stained against GFAP (green), MAP2 (red) and DAPI (blue). (c) Primary astrocytes stained against GFAP (green), NEUN (red) and DAPI (blue). (d) The enrichment of astrocytes was determined by flow cytometry of immunostained GLAST-positive cells isolated from the brains of 5 days old C57BL/6 J mice. Approximately 80% of the cells were GLAST-positive astrocytes after the separation on MACS columns.
biotin UTP, which generated multiple copies of biotinylated cRNA. The purity and concentration of the cRNA were checked using an ND-1000 Spectrometer. Quality cRNA was then hybridized using Illumina’s direct hybridization array kit (Illumina). Each cRNA sample (1.5 μg) was hybridized overnight to the MouseWG-6 BeadChip arrays (Illumina) in a multi-step procedure according to the manufacturer’s instructions; the chips were washed, dried and scanned on the BeadArray Reader (Illumina). Raw microarray data were generated using BeadStudio v3.0 (Illumina). Four biological replicates of the microarrays were prepared per experimental group. A total of 48 Illumina MouseWG-6 v2 microarrays (with probes for approximately 48,000 transcripts) were used in the experiment. To provide an appropriate balance in the whole dataset, groups were equally divided between the array hybridization batches.

Microarray data analysis

Microarray quality control was performed using BeadArray R package. The following parameters were checked: number of outliers, number of beads and percentage of probes detected. After background subtraction, the data were normalized using quantile normalization and then log2-transformed. The obtained signal was taken as the measure of mRNA abundance derived from the level of gene expression. Statistical analysis of the results was performed using two-way ANOVA (for the factors drug and cell type) followed by false discovery rate (FDR) correction for multiple testing. To obtain drug-versus-control comparisons, a t-test was used followed by FDR correction. All statistical analyses were performed in R software.

Cluster analysis

Hierarchical clustering was performed using the measure of correlation and complete linkage method. The cluster separation was performed based on up- and downregulation of transcripts. The functional annotation analysis tool DAVID was used to identify over-represented ontologic groups among the gene expression patterns and to group genes into functional classifications. For cell-type enrichment of mRNA, a recently published brain transcriptome database was used [13]. The identification of over-represented transcription factor binding sites was performed using the seqinspector database with default parameters [23].

Results

Differences in transcripts levels of steroid receptors between astroglial and neuronal primary cultures

We estimated levels of nuclear steroid receptors in neuronal and astroglial primary cultures using quantitative PCR (Fig. 2; upper panel). There was no difference between neurons and astrocytes in the expression of androgen receptor (Ar) and estrogen receptor 2 (Esr2) (p > 0.05). Neurons showed higher expression of mineralocorticoid receptor (Nr3c2) (p = 0.0005), progesterone receptor (Pgr) (p = 3.6 × 10−7), and estrogen receptor 1 (Esr1) (p = 1.1 × 10−6). Astrocytes showed higher levels of abundance of glucocorticoid receptor (Nr3c1) (p = 7.4 × 10−7). In addition, to compare mRNA levels between the receptors we used the available RNA-seq data from previous experiments by Piechota et al. [24] (Fig. 2; middle panel) and by Zhang et al. [13] (Fig. 2; lower panel). Consistent with the qPCR results, the previously published RNA-Seq studies by Piechota et al. [24] showed higher levels of expression of Nr3c2, Pgr and Esr1 in neurons and higher levels of expression of Nr3c1 in astrocytes. RNA-seq data published by Zhang et al. [13] were similar to qPCR results with two differences—astrocytes exhibited higher levels of Nr3c2, and mRNA of Esr1 was undetected.

Transcriptome alterations induced by steroid receptors agonists

We treated neuronal and astroglial primary cultures with dexamethasone (100 nM), aldosterone (200 nM), progesterone (200 nM) and estradiol (200 nM) for 4 h. For gene expression profiling, we used Illumina Mouse WG-6 v2 microarrays. By performing two-way ANOVA (with the factor cell type and drug) we identified 956 genes regulated by steroids (FDR < 1%). In astrocytes we found 381 genes altered by dexamethasone (t test, FDR 1%), 19 by aldosterone, 0 by progesterone (9 at FDR 20% threshold), and 0 by dihydrotestosterone and estradiol. In neurons we identified 0 genes regulated by dexamethasone (19 at FDR 20%), and 0 by aldosterone, progesterone, dihydrotestosterone and estradiol. We performed gene ontology analysis of genes regulated by dexamethasone (n = 381), aldosterone (n = 19) and progesterone (n = 9 at less restrictive threshold). The complete lists of regulated genes are presented in the supplementary materials (Additional file 1).

We performed functional analyses of genes regulated by dexamethasone, aldosterone and progesterone. Using the DAVID tool we looked for overrepresented gene ontology terms and molecular pathways. We found that genes upregulated by dexamethasone are functionally heterogeneous and include only a few overrepresented terms: positive regulation of cell proliferation (p = 1.0 × 10−3; FDR = 0.014; e.g. Bcl2l1, Cdkn1a, Trp63), negative regulation of inflammatory response (p = 0.0015; FDR = 0.21; Ada, Zfp36), cell junction (p = 0.012; FDR = 0.36; Amica1, Gjb6). The genes downregulated by dexamethasone are functionally related to the immune response:
toll-like receptor signaling pathway ($p = 5.7 \times 10^{-8}$; FDR = 4.1 \times 10^{-6}; Ccl3, Il1b, Tnf), cytokine–cytokine receptor interaction ($p = 5.2 \times 10^{-5}$; FDR = 1.9 \times 10^{-3}; Ccl12, Cxcr10), or immune response ($p = 1.2 \times 10^{-4}$; FDR = 0.098; Tlr2, Igh-6). We did not find any over-represented terms for genes regulated by aldosterone or progesterone.

We evaluated transcriptional mechanisms controlling gene expression using the seqinspector tool [23] that utilize previously published ChIP-seq experiments [12, 25]. Promoters of genes upregulated by dexamethasone in astrocytes were enriched in ChIP-seq signal for glucocorticoid receptor (SRA id: SRA027300, $p = 16 \times 10^{-10}$). Promoters of genes downregulated by dexamethasone in astrocytes have binding sites for E2F1 (GEO id: GSM881059, $p = 5.2 \times 10^{-5}$), REL (GEO id: GSM881134, $p = 4.7 \times 10^{-6}$), RELA (GEO id: GSM881114, $p = 6.0 \times 10^{-6}$), JUNB (GEO id: GSM881079, $p = 6.3 \times 10^{-6}$), STAT2 (GEO id: GSM881162, $p = 5.3 \times 10^{-5}$), IRF4 (GEO id: GSM881150, $p = 5.9 \times 10^{-5}$), NFKB1 (GEO id: GSM881154, $p = 1.2 \times 10^{-4}$). Promoters of genes
upregulated by aldosterone in astrocytes contain binding sites for glucocorticoid receptor (SRA id: SRA027300, \( p = 1.1 \times 10^{-14} \)). Genes regulated by progesterone in astrocytes have binding sites for BHLHE40 (GEO id: GSM912922, \( p = 2.3 \times 10^{-9} \)) and glucocorticoid receptor (SRA id: SRA027300, \( p = 8.7 \times 10^{-9} \)).

**Gene expression changes induced by corticosterone**

We compared transcriptional alterations induced by dexamethasone and aldosterone to endogenous non-specific glucocorticoid and mineralocorticoid receptors agonist, corticosterone. The effects of corticosterone on gene expression were measured in neurons and astrocytes in time and dose dependent manner by using quantitative PCR. From the list of genes detected by microarray profiling we selected three previously known glucocorticoid-receptor-dependent transcripts (\( Fkbp5 \), \( Zbtb16 \) and \( Sgk1 \)) (Fig. 3). For statistical analysis we used three way ANOVA for cell type, dose and time factors. Interaction between factors was statistically significant \( (p < 0.05) \) for all of the transcripts examined. Tukey HSD test was used as a post hoc test. All inspected transcripts were significantly upregulated in astrocytes (at doses of 50 nM and 200 nM) in both 4 h and 8 h time points. None of the inspected transcripts were significantly regulated in neuronal cells.

**Glial enrichment of dexamethasone induced gene expression alterations in neurons**

We observed that the genes induced in neurons (top 20, \( t \) test FDR < 26%) were expressed at higher levels in astrocytes \( (p = 0.0017, t \) test between expression levels for controls in neurons and astrocytes). Using the top 100 genes identified by two-way ANOVA in the whole experiment and compared fold induction of astroglia and neuron-specific genes in dexamethasone treated neurons (Fig. 4). In neuronal primary cultures, we observed higher induction of dexamethasone-induced astroglia enriched genes than dexamethasone-induced neuron enriched genes \( (p = 0.032, t \) test between fold changes in gene expression) (Fig. 5).

**Discussion**

The most remarkable observation of this study was the weak, or absent response of neurons to hormonal stimulus. As has previously been suggested, the main component of transcriptional response to adrenal steroids is not neuronal but astroglial \([8, 26, 27]\). This hypothesis was based on astrocyte-specific gene enrichment (higher level of expression) among glucocorticoid inducible transcripts \([26]\). In our previous studies, we have also shown that an inducible form of \( Sgk1 \) is present in astrocytes and not in neurons \([18]\). In that study, we found that, unlike astrocytes, neurons are not transcriptionally responsive to dexamethasone treatment. We hypothesized that
in the previous experiments, the lack of transcriptional response of neurons to dexamethasone might have been caused by cell culture conditions and low concentration of dexamethasone (10 nM). Thus, in this study neurons and astrocytes were cultured in the same manner for the final 3 days. However, despite all those previous indications, we were startled by the low induction of gene expression in neurons. The most unexpected result was the lack of response of neurons to progesterone, despite the high expression of progesterone receptor [13] and the promotion of neuronal survival by this steroid [28, 29]. The presented in vitro study has several limitations. The model may not accurately reflect the effect of steroids on gene expression in neurons in the brain. It is possible that neurons isolated from embryonic tissue are subsensitive to steroids. Such a mechanism may insulate the brain from developmental interference from external steroids such as xenobiotic or maternally-generated steroids. Neonatal neurons cultured for 7 days are not fully developed and may have a lower number of functional synapses compared to mature cells. Moreover, our study was designed to detect changes with twofold or higher, with a power of >50%. Thus, smaller changes in mRNA abundance levels in neurons might have gone undetected.

Neither neurons nor astrocytes responded to estradiol or dihydrotestosterone, likely because of low levels of these receptors in our cultures. Although these steroids have not acted transcriptionally in our case, they might act on membrane channels and receptors such as GABAA [30]. Although the astrocytes express far fewer progesterone receptors [13] than do neurons, they responded to progesterone transcriptionally. Nevertheless, this response was much weaker than those caused by dexamethasone or aldosterone. Astrocytic response to dexamethasone and aldosterone were qualitatively similar. This pattern of transcriptional activation was also detected in neuronal cells. However, the profile of changes indicates the very small number of contaminating astrocytes as a source of gene expression alterations. These results suggest that in our experiment response of neuronal cells to steroid hormones is repressed.

The mechanism of steroid nuclear receptor repression in neurons is intriguing. Our first hypothesis was focused on non-active isoforms of these receptors. We
verified this speculation by reviewing the RNA-seq brain
gene expression atlas [13] and FANTOM5 cage data [31].
In fact, the transcriptional isoform of Pgr expressed in
neurons is shorter and has not been previously anno-
tated in the Ensembl database. However, neurons and
astrocytes express the same transcriptional isoforms of
Nr3c1 and Nr3c2, which disproves this conjecture. Our
second hypothesis concerned the absence of nuclear
receptor binding sites in neurons. The chromatin avail-
ability predetermines the binding sites of glucocorticoid
receptor [12]. Nonetheless, such a complete blockade is
rather unlikely. Our third hypothesis involves an imbal-
ance between coactivators and corepressors of steroid
receptor [12].

The genes regulated in astrocytes in vitro by dexam-
ethasone overlap with other studies of transcriptional
alterations induced by steroids in vivo. Nine out of the
top ten genes regulated by dexamethasone in mouse
hypothalamus are also regulated in primary astro-
cytes (i.e. Gadd45 g, Sgk1, Pdk4, Nfkbia, Plekhf1, Errfi1,
Arrdc2, Dusp1 and Bcl6) [37]. Similarly, multiple genes
regulated by corticosterone in the rat hippocampus
were identified in the presented study (e.g. Bcl6, Fkbp5,
Gjb6, Errfi1, Pdk4) [38]. The genes upregulated by dexam-
ethasone and aldosterone in astrocytes are function-
ally heterogeneous, which suggests diverse molecular
mechanisms of action. One of these mechanisms may
be related to the regulation of energy supply to neuronal
cells. Genes involved in this process include a major glu-
cos transporter in the mammalian blood–brain barrier
Slc2a1 [39], which was upregulated after dexamethasone
or aldosterone treatment. Another gene Pdk4 maintains
pyruvate dehydrogenase in the phosphorylated, inactive
state and shunts pyruvate to form lactate in astrocytes
[13]. Dexamethasone also upregulates the putative sugar
transporter S1c45a3 [40]. Another set of regulated genes
represents control of a negative feedback on the action
of steroids. The genes involved in this process include
Fkbp5 [41] and Hspa1b [42]. Astrocytes also show
upregulation of the cell adhesion molecules Amica1 and
Gjb6, which might influence the extracellular matrix and
support neuronal plasticity. The genes downregulated
by dexamethasone are homogeneous and are involved
in inflammatory response. Dexamethasone can effec-
tively attenuate the inflammatory response to wounding,
reducing neuronal and glial scarring in the vicinity of the
wound [43].

Conclusions
Our study sheds new light on the mechanisms of action
of steroid hormones in the central nervous system. The
results show that glial cells constitute a primary target for
the rapid transcriptional action of steroids. Identification
of genes that mediate effects of adrenal steroids on astro-
cytes revealed novel molecular mechanisms involved in
regulation of brain metabolism and plasticity. The unex-
pectedly low transcriptional response of neuronal cells to
steroids provides rationale for further in vivo studies on
this issue.

Additional files

Additional file 1. Lists of regulated genes. The complete list of genes
regulated by dexamethasone, aldosterone, progesterone, dihydrotestos-
erone and estradiol. The list includes p values, corrected p values and
log2 ratios for all pairwise comparisons.

Additional file 2. Cell expression analysis of glucocorticoid receptor
cofactors.

Abbreviations
PG: progesterone; DHT: dihydrotestosterone; ES: estradiol; FPKM: fragments
per kilobase of exon per million fragments mapped.

Authors’ contributions
MP designed the experiments. SG and DJ performed the experiments. MP
and MK drafted manuscript. MP, MK and JF analyzed data. RP and BZ provided
valuable guidance and discussion. All authors interpreted results and edited
the manuscript.

Competing interests
The authors declare that they have no competing interests.

Availability of data and materials
The raw expression data is available on the NCBI Gene Expression Omnibus
(GEO) under Accession Number GSE78281.

Ethics approval and consent to participate
The animal protocols used in the study were approved by the local Bioethics
Commission at the Institute of Pharmacology, Polish Academy of Sciences
(Krakow, Poland).

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References
1. McEwen BS. Stress, adaptation, and disease. Allostasis and allostatic load. Ann N Y Acad Sci. 1998;840:33–44 (Wiley Online Library).
2. Herman JP, Cullinan WE. Neurocircuity of stress: central control of the hypothalamo–pituitary–adrenocortical axis. Trends Neurosci. 1997;20:78–84 (Elsevier).
3. Fehm HL, Benkowitsch R, Kern W, Fehm-Wolfsohn G, Pauschinger P, Born J. Influences of corticosteroids, dexamethasone and hydrocortisone on sleep in humans. Neuropsychobiology. 1986;16:198–204 (Karger.com).
4. Erickson K, Drevets W, Schulkin J. Glucocorticoid regulation of diverse cognitive functions in normal and pathological emotional states. Neurosci Biobehav Rev. 2003;27:233–46 (Elsevier).
5. Rozendaal B. Glucocorticoids and the regulation of memory consolidation. Psychoneuroendocrinology. 2000;25:213–38 (Elsevier).
6. McEwen BS. Mood disorders and allostatic load. Biol Psychiatry. 2003;54:200–7 (Elsevier).
7. Korostynski M, Piechota M, Dzbek J, Mlynarski W, Szklarczyk K, Ziołkowska B, et al. Novel drug-regulated transcriptional networks in brain reveal pharmacological properties of psychotropic drugs. BMC Genom. 2013;14:606 (biomedcentral.com).
8. Piechota M, Korostynski M, Soleczi W, Gieryk A, Slezak M, Blecki W, et al. The dissection of transcriptional modules regulated by various drugs of abuse in the mouse striatum. Genome Biol. 2010;11:R48.
9. Guerry JD, Hastings PD. In search of HPA axis dysregulation in child and adolescent depression. Clin Child Fam Psychol Rev. 2011;14:435–60.
10. Bitran D, Shiell M, McLeod M. Arxiolystic effect of progesterone is mediated by the steroidor allopregnanolone at brain GABAAR receptors. J Neuroendocrinol. 1995;7:171–7 (Wiley Online Library).
11. McEwen BS, Woolley CS. Estradiol and progesterone regulate neuronal structure and synaptic connectivity in adult as well as developing brain. Exp Gerontol. 1994;29:431–6 (Elsevier).
12. John S, Sabo PJ, Thurman RE, Sung M-H, Biddle SC, Johnson TA, et al. Chromatin accessibility pre-determines glucocorticoid receptor binding patterns. Nat Genet. 2011;43:264–8 (nature.com).
13. Zhang Y, Chen K, Sloan SA, Bennett ML, Scholze AR, O’Keeffe S, et al. An RNA-sequecing transcription and splicing database of glia, neurons, and vascular cells of the cerebral cortex. J Neurosci. 2014;34:11929–47.
14. Oitzl MS, Fluttet M, de Kloet ER. The effect of corticosterone on reactivity to spatial novelty is mediated by central mineralocorticoid receptor. Eur J Neurosci. 1994;6:1072–9 (Wiley Online Library).
15. Rundlett SE, Miesfeld RL. Quantitative differences in androgen and glucocorticoid receptor DNA binding properties contribute to receptor-selective transcriptional regulation. Mol Cell Endocrinol. 1995;109:1–10.
16. Haynes LE, Griffiths MR, Hyde RE, Barber DJ, Mitchell IJ. Dexamethasone induces limited apoptosis and extensive sublethal damage to specific subregions of the striatum and hippocampus: implications for mood disorders. Neuroscience. 2001;104:57–69.
17. Op de Macks ZA, Gunther Moor B, Overgaauw S, Güroğlu B, Dahl RE, Crone EA. Testosterone levels correspond with increased ventral striatum activation in response to monetary rewards in adolescents. Dev Cogn Neurosci. 2011;1:506–16.
18. Slezak M, Korostynski M, Gieryk A, Golda S, Dzbek J. Astrocytes are a neural target of morphine action via glucocorticoid receptor-dependent signaling. Glia [Internet]. 2013. doi:10.1002/glia.23828 (Wiley Online Library).}

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23. Plechota M, Korostynski M, Ficek J, Tomski A, Przewlocki R. Sequinspec-tror: position-based navigation through the ChIP-seq data landscape to identify gene expression regulators. BMC Bioinform. 2016;17:499.
24. Plechota M, Golda S, Ficek J, Jantas D, Przewlocki R, Korostynski M. Regulation of alternative gene transcription in the striatum in response to antidepressant drugs. Neuropharmacology. 2015;99:328–36.
25. Stamatoyannopoulos JA, Snyder M, Hardison R, Ren B, Gingeras T, Gilbert DM, et al. An encyclopedia of mouse DNA elements (Mouse ENCODE). Genome Biol. 2012;13:418 (BioMed Central Ltd).
26. Carter BS, Meng F, Thompson RC. Glucocorticoid treatment of astrocytes results in temporally dynamic transcriptorpe regulation and astrocyte-enriched mRNA changes in vitro. Physiol Genom. 2012;44:1188–200.
27. Carter BS, Hamilton DE, Thompson RC. Acute and chronic glucocorticoid treatments regulate astrocyte-enriched mRNAs in multiple brain regions in vivo. Front Neurosci. 2013;7:139.
28. Charalampopoulos I, Remboutsika M, Margsioris AN, Gravanis A. Neuro-steroids as modulators of neurogenesis and neuronal survival. Trends Endocrinol. 2008;19:300–7 (Metab. Elsevier).
29. Zhang Z, Yang R, Zhou R, Li L, Sokabe M, Chen L. Progesterone promotes the survival of newborn neurons in the dentate gyrus of adult male mice. Hippocampus. 2010;20:402–12.
30. Majewska MD, Harrison NL, Schwartz RD, Barker JL, Paul SM. Steroid hormone metabolites are barbiturate-like modulators of the GABA receptor. Science. 1986;232:1004–8 (sciencemag.org).
31. FANTOM Consortium and the RIKEN PMI and CLST (DGT), Forrest ARR, Kawaji H, Rehli M, Baille J, de Hoon M, et al. A promoter-level mammalian expression atlas. Nature. 2014;507:462–70.
32. Zhang X, Jeyakumar M, Petkovsk S, Bagchi MK. A nuclear receptor corepressor modulates transcriptional activity of antagonist-occupied steroid hormone receptor. Mol Endocrinol. 1998;12:513–24.
33. Kumar R, Gururaj AE, Vadlamudi RK, Rayala SK. The clinical relevance of steroid hormone receptor corepressors. Clin Cancer Res. 2005;11:2822–31.
34. Gao X, Nawaz Z. Progesterone receptors—animal models and cell signaling in breast cancer: role of steroid receptor coactivators and corepressors of progesterone receptors in breast cancer. Breast Cancer Res. 2002;4:182–6.
35. Saradbjisirnang RA, Joels M, de Kloet ER. Glucocorticoid pulsatility and rapid corticosteroid actions in the central stress response. Physiol Behav. 2012;106:73–80 (Elsevier).
36. Lösel R, Wehling M. Nongenomic actions of steroid hormones. Nat Rev Mol Cell Biol. 2003;4:46–56 (nature.com).
37. Sato H, Horikawa Y, Izuoka K, Sakurai N, Tanaka T, Shihaara N, et al. Large-scale analysis of glucocorticoid target genes in rat hypothalamus. J Neurochem. 2008;106:803–14.
38. Datson NA, van den Oever JME, Korobko OB, Magarinos AM, de Kloet ER, McEwen BS. Previous history of chronic stress changes the transcriptional response to glucocorticoid challenge in the dentate gyrus region of the male rat hippocampus. Endocrinology. 2013;154:261–72.
39. Zheng P-P, Romme E, van der Spek PJ, Dirven CMF, Willemsen R, Kros JM. Glut1/SLC2A1 is crucial for the development of the blood-brain barrier in vivo. Ann Neurol. 2010;68:835–44.
40. Vitáska O, Wieczorek H. The SLC24 gene family of putative sugar transporters. Mol Aspects Med. 2013;34:655–60.
41. Reynolds PD, Ruan Y, Smith DF, Scammell TG. Glucocorticoid resistance in the squirrel monkey is associated with overexpression of the immunophi- lin FBP51. J Clin Endocrinol Metab. 1999;84:663–9.
42. Kirschke E, Goswami D, Southworth D, Griffin PR, Agard DA. Glucocorti-coid receptor function regulated by coordinated action of the Hsp90 and Hsp70 chaperone cycles. Cell. 2014;157:1685–97.
43. Zhong Y, Bellamkonda V. Dexamethasone-coated neural probes elicit attenuated inflammatory response and neuronal loss compared to uncoated neural probes. Brain Res. 2007;1148:15–27.