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Transcriptome profiles of corticosterone-induced cytotoxicity reveals the involvement of neurite growth-related genes in depression

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ABSTRACT

Corticosterone (CORT), the main HPA-axis glucocorticoid hormone in rodents, is involved in the regulation of animal stress responses. However, the neural mechanisms underlying the effects of corticosteroids on depression are yet to be elucidated. We found that fluoxetine reversed neurite growth inhibition induced by CORT in PC12 cells, a widely used model system for neurobiological and neurotoxicological studies. **Transcriptome profiling showed that 1,609 genes were up-regulated, whereas 1,764 genes were down-regulated significantly in the CORT group in comparison with the Control group. Of them, the expression of 589 DEGs was reversed after fluoxetine treatment, and genes related to cell morphogenesis, neurite growth, and immune function were involved in the neuroprotective effect of fluoxetine against CORT.** Furthermore, expression of neurite growth-related genes, such as such as Calpain 2 *(Capn2)*, vesicle-associated membrane protein 7 (*Vamp7)* and C-type natriuretic peptide (*Cnp*), altered in a brain region- or treatment-specific manner in the animal models of depression. Therefore, the interaction between stress, glucocorticoids, and neurite growth inhibition may be a candidate pathophysiology underlying major depressive disorder (MDD), and the identification of *Capn2, Vamp7* and *Cnp* might provide insight into treatment of MDD.

1. Introduction

Environmental stress is a risk factor for various mental disorders, especially for depression. Glucocorticoid hormones are pivotal neuroendocrine signals, and play essential roles in the regulation of physiological response to stress (Gourley et al., 2013). Corticosterone, the main hypothalamic-pituitary-adrenal (HPA)-axis glucocorticoid hormone in rodents, was shown to be involved in the regulation of animal stress responses. Elevated basal corticosterone concentration, caused by hyperactivity of the HPA axis and glucocorticoid receptor (GR) dysfunction, was considered as one of the major features of depression in both experimental animals and patients (Blackburn-Munro and Blackburn-Munro, 2001; Gong et al., 2015). However, serum corticosterone concentration showed different dynamics under different physiological or stressful conditions (Liu et al., 2013; Wu et al., 2012).

Glucocorticoid hormones play an important role in neuronal system development, metabolic regulation, and stress response (Colich et al., 2015; Roszkowski et al., 2016; Wang et al. 2015; Zhao et al., 2015). Additionally, they have extensive effects on neural activity through the genomic regulation of gene transcription and other non-genomic pathways, which are mediated by their mineralocorticoid (MR) and glucocorticoid (GR) receptors (Joels et al., 2012). In contrast, previous studies reported that high corticosterone concentrations and long-lasting corticosterone treatment could induce neuronal cell damage (Du et al., 2009; Marasco et al., 2016; Zou et al., 2016). Additionally, it was reported that glucocorticoids could significantly increase the amount of mitochondrial DNA, and reduce the length of telomeres in response to stress in an experimental animal model (Tomiyama et al., 2012).

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Furthermore, an increase in circulating corticosterone concentration may lead to changes in multi-gene expression levels, resulting in a decrease in the concentration of physiologically important neurotropic factors and causing multi-systemic disorders (Mpofana et al., 2016; Roszkowski et al., 2016; Wong et al., 2008). However, the neural mechanisms underlying the effects of corticosteroids on depression are yet to be elucidated.

The PC12 cell line is widely used as a model system for both the glucocorticoid-induced impairment of neuronal cells and their underlying molecular mechanisms (Terada et al., 2014) due to the particularly abundant distribution of glucocorticoid receptors in the cells (Fujita et al., 1989). Previous studies investigated the effects of glucocorticoids on neurite outgrowth in PC12 cells (Pollock et al., 1990; Terada et al., 2014). Our previous research also confirmed that corticosterone significantly affected PC12 cell viability through the alternation of multi-gene expression levels in intracellular MAPK pathways (Li et al., 2016). To further explore the neural mechanisms underlying the effects of corticosteroids on depression, we performed transcriptome profiling to test the effect of fluoxetine, a wildly used antidepressant, on neurite growth inhibition induced by CORT in PC12 cells. Additionally, we examined the expression changes of target genes (chosen by bioinformatics) in two animal depression models induced by either unpredicted chronic mild stress or chronic corticosterone treatment.

2. Materials and methods

total of 24 ten-week-old male Sprague Dawley (SD) rats (Beijing Vital River Laboratory Animal Technology Co., Ltd, Beijing, China) were used for all experiments, which were strictly performed in accordance with the Guideline for Care and Use of Laboratory Animals of the Chinese Academy of Sciences. All protocols were approved by the Review Board of the Institute of Psychology, Chinese Academy of Sciences.

2.1. Drugs and reagents

Fluoxetine (CAS 56296-78-7; Melonepharma, Dalian, China) was dissolved in dimethyl sulfoxide (DMSO) to create a stock solution, while corticosterone and Cell Counting Kit-8 (CCK-8) were purchased from SIGMA-ALDRICH (Cat No. C2505; Saint Louis, MO, USA) and Beyotime (Cat. No. C0038; Beyotime Institute of Biotechnology, Haimen, China), respectively.

2.2. Cell culture

PC12 cells were obtained from the Cell Bank of the Chinese Academy of Sciences (Beijing, China) and maintained in the Dulbecco's modified Eagle's medium (DMEM) (Life Technologies, Grand Island, NY, USA) supplemented with 5% of fetal bovine serum (FBS) (Life Technologies, Grand Island, NY, USA), 5% of horse serum (Life Technologies, Grand Island, NY, USA), 100 μg/ml of streptomycin, and 100 U/ml of penicillin in a water-saturated atmosphere of 5% CO_2 at 37 °C.

2.3. Cell treatment

PC12 cells were seeded into either 96-well dish for cell viability examination or 60-mm dish for RNA extraction with 3×10^3 cells/well and 1×10^6 cells/well, respectively, and were cultured in serum-free medium for 12 h prior to drug treatment. Additionally, cells were treated with 50 uM CORT and various concentrations of fluoxetine (0–100 ng/ml) for 24 h, while cell viability was measured by CCK-8 assay. **The CORT concentration was chosen according to our previous study which could reduce both live cell numbers and cellular neurite outgrowth (Li et al., 2016).** PC12 cells were divided into Control, CORT, and CORT (50 uM) plus fluoxetine (5, 10, 100 ng/ml) treatment groups. The cells in both of the control and treatment groups were harvested after 24 h following either CORT or fluoxetine treatment.

2.4. RNA sequencing (RNA-seq)

Total RNA of the control and drug-treated PC12 cells was extracted using Trizol (Life Technologies, Grand Island, NY, USA). Both, the quantity and the quality, of the extracted RNA were evaluated using the NanoDrop spectrophotometer (Thermo Scientific, Wilmington, DE, USA) and Agilent 2100 Bioanalyzer with calculation of RNA integrity number (RIN) (Agilent Technologies, Santa Clara CA, USA), respectively. All RNA samples were applied for the next generation RNA sequencing when the sample RIN $>$ 7 and the total RNA quantity $>$ 400 ng. Nine sequencing libraries (3 Control groups, 3 CORT and 3 CORT plus 100 ng/ml fluoxetine treatment groups) were constructed with the NEBNext Ultra RNA Library Prep Kit for Illumina with poly(A) messenger RNA selection (New England Biolabs, Ipswich, MA, USA), according to the manufacturer's instructions. Thereafter, the libraries were used to build clusters on the Illumina cell flow according to the manufacturer's protocol. Finally, sequencing was performed using the Illumina HiSeq 2500 System (Illumina, San Diego, CA, USA) with a 150-bp paired-end sequencing strategy.

2.5. Bioinformatic processing of the RNA-seq data

The quality of raw sequencing reads was evaluated by using the FastQC package, and then Cutadapt was used to remove adapters and low quality reads (Martin, 2011). The clean reads were then mapped into the rat reference genome and transcriptome downloaded from the UCSC Genome Browser (http://www.genome.ucsc.edu) (rn5 assembly) through the Tophat2 (Trapnell et al., 2009). Only uniquely mapped reads were used in this analysis. Fragments Per Kilobase of sequence per Million mapped reads (FPKM) were used to normalize the number of aligned reads by the size of the gene and the total number of mapped reads. Additionally, the changes in gene expression between different groups were analyzed using the Cufflinks 2.0 package (Trapnell et al., 2012). Genes with FDR (False Discovery Rate) value $<$ 0.05 were referred to as differentially expressed genes (DEGs).

We further identified the statistically enriched functional terms from the public databases of the Gene Ontology (GO) (http://geneontology.org/); functional terms with a FDR value $<$ 0.05 represented a statistically significant enrichment.

2.6. Validation of differentially expressed genes

cDNA was synthesized from 1 μg of total RNA (template) using Superscript III (Invitrogen, CA, USA). The validation of differentially expressed genes (DEGs) was determined by real-time quantitative PCR using the SYBR Green Mix (CWBIO, Beijing, China) **on Applied Biosystems ABI 7500 (Applied Biosystems, CA, USA)**. PCR conditions were: 95 °C for 10 min, 40 cycles at 95 °C for 10 s, 60 °C for 60 s, and signal detection for 10 s. The dissociation curve analysis was performed at the end of each run, whereas the relative mRNA expression was determined by the standard $^{\Delta\Delta}$Ct method using the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as a normalization control. **The primers for target genes design by software PerlPrimer (Marshall, 2004) were listed in Table 1, and specificity of each primer was validated using gel electrophoresis**.

2.7. Chronic unpredicted mild stress (CUMS)

The time schedule followed for the CUMS procedure was previously reported in different studies (Chen et al., 2015; Pan et al., 2014). All rats were isolated into a single cage and subject to a variety of mild

Table 1
Primers used for target genes.

Gene	Primers (5′→3′) Forward	Reverse
Cnp	CCCAACAGGATGTGGTGAGG	AACTGCAGCTCCTGCTCATT
Capn2	AGTTCTAGCCAAGCGCGAAG	TCCACGTCGATCTCTCGGTA
Vamp7	TTCTCGGGCCTTCGGTTTTT	GATGCTTCAGTTGTGCAGCC

stressors, including cage tilting for 24 h, swimming in cold water at 4 °C for 5 min, swimming in hot water at 45 °C for 5 min, fasting for 48 h, water deprivation for 24 h, shaking for 10 min, nip tail for 1 min, wet bedding for 24 h, and inversion of the light/dark cycle. Rats were exposed to one of such stressors each day. The stress procedure lasted for eight weeks prior to behavioral testing, with food deprivation for 20 h representing the final stressor.

2.8. Chronic corticosterone (CORT) treatment

CORT was emulsified in propylene glycol (Fisher Scientific, Pittsburgh, PA, USA). Subsequently, either CORT or the vehicle was administered subcutaneously (s.c.) once per day, at a dose of 20 mg/kg of rats' body weight. Injections were administered between 09:00 and 11:00 for 21 consecutive days. The dose was selected based on previous studies which reported similar doses to be effective in inducing depression-like behavior in rats (Rosa et al., 2014).

2.9. Data analysis

All the statistical analyses were performed with the GraphPad Prism 6.0 software. Comparisons of means of multiple groups with each other or with the Control group were performed using one-way ANOVA followed by Tukey's post-hoc tests. The means of relative gene expression between the two groups were compared with a two-tailed Student's *t*-test. Statistical significance was set at $P < 0.05$.

3. Results

3.1. Fluoxetine reversed corticosterone-induced cytotoxicity and inhibition of neurite growth in PC12 cells

Following the 24-h treatment of PC12 cells with 50 μM of CORT and various concentrations of fluoxetine (0–100 ng/ml), cell viability was remarkably reduced, with a significant difference between the Control and the CORT treatment groups ($P < 0.01$). In contrast, co-treatment with fluoxetine enhanced cell viability at the dose of 100 ng/ml ($P < 0.05$) (Fig. 1). Considering that the protective effect of fluoxetine was observed at 100 ng/ml, cells treated with such a concentration of fluoxetine were used for transcriptome sequencing. Additionally, the microscopic observation showed that the neurites of CORT-treated PC12 cells were significantly shorter or even disappeared when compared to that of control cell, which was alleviated by co-treatment with fluoxetine (Fig. 2).

3.2. Quality assessment of the sequenced data

We obtained 46,306,299 to 50,181,829 short reads of 150 bp from each replicate from the CORT-treated, the CORT- plus fluoxetine-treated, and Control groups. Of these, 90.34% to 91.78% were successfully aligned to a reference gene database (UCSC, version rn5) by TopHat (Trapnell et al., 2009) (Supp. Table 1).

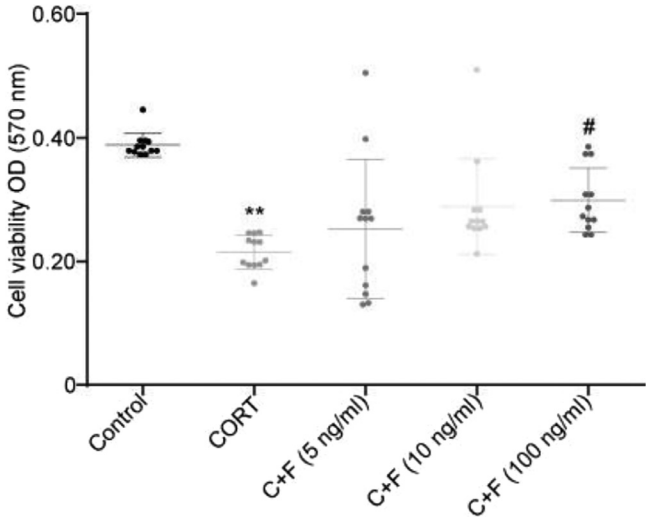

Fig. 1. Fluoxetine alleviated corticosterone (CORT)-induced cytotoxicity. Cell viability in the Control, CORT (50 uM) and co-treatment of 50 uM CORT (C) with fluoxetine (F) groups. Data is expressed as the mean ± SEM (***n* = 12 for each group**). $^{**}P < 0.01$ compared with the Control group; $^{\#}P < 0.05$ compared with the CORT group.

3.3. CORT-induced expression changes were specifically reversed by fluoxetine

The analysis showed that when compared to the Control group, 1609 genes were up-regulated, whereas 1764 genes were down-regulated significantly in the CORT group. Of them, the expression of 589 DEGs was reversed after fluoxetine treatment). Through functional enrichment analysis, 23 groups were enriched for CORT-induced-DEGs whose expression changes can be reversed by fluoxetine treatment (Tables 2 and 3). The most significantly enriched GO items were related to cell morphogenesis (GO:0001763 ~ morphogenesis of a branching structure), neurite growth (GO:0031143 ~ pseudopodium), immune function (GO:0010934 ~ macrophage cytokine production, GO:0016236 ~ macroautophagy), and cell growth/apoptosis (GO:0016071 ~ mRNA metabolic process, GO:0006397 ~ mRNA processing, GO:0008380 ~ RNA splicing, GO:0006281 ~ DNA repair and so on). It suggests that genes related to cell morphogenesis, neurite growth, and immune function might be involved in the neuroprotective effect of fluoxetine against CORT.

3.4. Validation of the expression changes of pseudopod extensions-related genes by qRT-PCR

Consistent with the morphological changes in PC12 cells, we found that three differentially expressed genes (*Capn2, Vamp7* and *Cnp*) were involved in pseudopod extensions (GO:0031143 ~ pseudopodium), which were essential for neurite outgrowth of PC12 cell (Dharmawardhane et al., 1999). These genes were selected for validating the RNA-seq results by RT-qPCR. The RT-qPCR results showed that the significant alteration induced by CORT could be reversed by fluoxetine treatment, which coincided with the results obtained from RNA-sequencing except for *Cnp* (Supp. Fig. 1).

3.5. Chronic CORT treatments changed the expression of Capn2, Vamp and Cnp in various brain regions

Our unpublished data found that animals showed depressive like behavior after chronic CORT treatments. We further tested the effects of chronic CORT treatments on the expression of *Capn2, Vamp* and *Cnp* in various HPA axis function and stress-related brain regions. The results showed that *Capn2* increased in the prefrontal cortex (PFC) ($P < 0.05$),

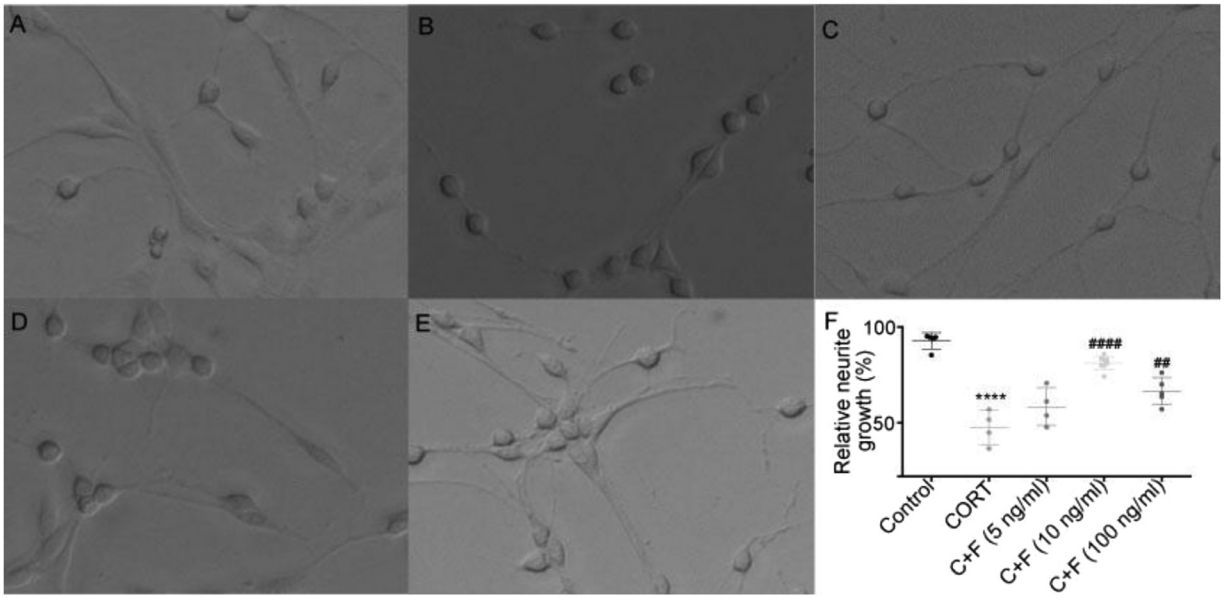

Fig. 2. Fluoxetine alleviated neurite growth inhibition induced by corticosterone (CORT) in PC12 cells. Representative cell morphology in the Control group (A), CORT group (B), and co-treatment of 50 uM CORT with 5 ng/ml (C), 10 ng/ml (D) and 100 ng/ml (E) fluoxetine, respectively. (F) Relative neurite growth is expressed as the mean ± SEM in the Control, CORT and co-treatment of CORT with fluoxetine groups (***n* = 4–8 for each group**). $^{****}P < 0.0001$ compared with the Control group; $^{\#\#\#\#}P < 0.0001$ compared with the CORT group; $^{\#\#}P < 0.01$ compared with the CORT group.

Table 2
Gene Ontology annotations of CORT-upregulated genes whose expression changes can be reversed by fluoxetine treatment.

Terms	%	Adjusted*P* Value	Genes
GO:0001763 ~ morphogenesis of a branching structure	5.56	3.6E-3	Cav3, Clic4, Dlg5, Fat4, Grb2, Hoxb13, Plxnd1, Rere, Six4, Snai2, Wnt5a
GO:0003151 ~ outflow tract morphogenesis	11.1	5.7E-3	Dhrs3, Fzd1, Gata6, Parva, Plxnd1, Wnt5a
GO:0061138 ~ morphogenesis of a branching epithelium	5.43	10.0E-3	Cav3, Clic4, Dlg5, Fat4, Grb2, Hoxb13, Plxnd1, Six4, Snai2, Wnt5a
GO:0010935 ~ regulation of macrophage cytokine production	30.0	24.0E-3	Irak3, Tlr4, Wnt5a
GO:0060411 ~ cardiac septum morphogenesis	10.9	27.0E-3	Dhrs3, Fzd1, Gata6, Parva, Wnt5a
GO:0010934 ~ macrophage cytokine production	27.27	32.0E-3	Irak3, Tlr4, Wnt5a
GO:0003279 ~ cardiac septum development	7.79	40.0E-3	Dhrs3, Fzd1, Gata6, Parva, Plxnd1, Wnt5a
GO:0016236 ~ macroautophagy	4.17	44.0E-3	Atg14, Fam134b, Gabarapl2, Krcc1, Larp1b, Map1a, Pik3c3, Rubcn, Slc35b3, Trim13, Vdac1]
GO:1903008 ~ organelle disassembly	4.95	44.0E-3	Atg14, Fam134b, Fis1, Gabarapl2, Krcc1, Larp1b, Map1a, Slc35b3, Vdac1
GO:0005912 ~ adherens junction	4.30	820.0E-6	Adam9, Ajap1, Bcar1, Capn2, Cav2, Cd44, Cspg4, Dcaf6, Dlc1, Dlg5, Fzd1, Layn, Lima1, Parva, Ppfibp1, Rdx, Rpl30
GO:0005925 ~ focal adhesion	4.60	1.3E-3	Adam9, Bcar1, Capn2, Cav2, Cd44, Cspg4, Dcaf6, Dlc1, Fzd1, Layn, Lima1, Parva, Ppfibp1, Rdx, Rpl30
GO:0005924 ~ cell-substrate adherens junction	4.56	1.4E-3	Adam9, Bcar1, Capn2, Cav2, Cd44, Cspg4, Dcaf6, Dlc1, Fzd1, Layn, Lima1, Parva, Ppfibp1, Rdx, Rpl30
GO:0031143 ~ pseudopodium	23.8	53.0E-3	Capn2, Cnp, Vamp7

Table 3
Gene Ontology annotations of CORT-downregulated genes whose expression changes can be reversed by fluoxetine treatment.

Terms	%	Adjusted*P* Value	Genes
GO:0016071 ~ mRNA metabolic process	5.46	27.0E-6	Bag4, Clasrp, Cpsf6, Cwc22, Dxo, Eif4a3, Exosc8, Hnrnpc, Hnrnpl, Hnrnpr, Leo1, Lsm11, Lsm7, Lsm8, Magoh, Nelfe, Phf5a, Ppil3, Snrnp70, Snrpd2l, Srsf4, Upf1, Ybx1
GO:0006397 ~ mRNA processing	5.25	980.0E-6	Clasrp, Cpsf6, Cwc22, Eif4a3, Hnrnpc, Hnrnpl, Leo1, Lsm11, Lsm7, Lsm8, Magoh, Nelfe, Phf5a, Ppil3, Snrnp70, Snrpd2l, Srsf4, Ybx1
GO:1903311 ~ regulation of mRNA metabolic process	9.01	1.1E-3	Bag4, Eif4a3, Hnrnpc, Hnrnpl, Hnrnpr, Leo1, Magoh, Nelfe, Snrnp70, Srsf4
GO:0008380 ~ RNA splicing	5.15	6.5E-3	Clasrp, Cwc22, Eif4a3, Hnrnpc, Hnrnpl, Lsm7, Lsm8, Magoh, Phf5a, Ppil3, Snrnp40, Snrnp70, Snrpd2l, Srsf4, Ybx1
GO:0006281 ~ DNA repair	4.46	20.0E-3	Ercc1, Ercc2, Faap100, Mad2l2, Msh5, Ns5atp9, Parpbp, Polk, Rad1, Rad50, Rad51b, Rpa3, Trim28, Ube2n, Wrnip1, Xrcc1
GO:1903312 ~ negative regulation of mRNA metabolic process	13.16	32.0E-3	Bag4, Hnrnpc, Hnrnpl, Nelfe, Srsf4
GO:0000377 RNA splicing	5.39	41.0E-3	Cwc22, Eif4a3, Hnrnpc, Hnrnpl, Lsm7, Lsm8, Magoh, Phf5a, Snrnp70, Snrpd2l, Srsf4
GO:0000398 ~ mRNA splicing, via spliceosome	5.39	41.0E-3	Cwc22, Eif4a3, Hnrnpc, Hnrnpl, Lsm7, Lsm8, Magoh, Phf5a, Snrnp70, Snrpd2l, Srsf4
GO:0000375 ~ RNA splicing, via transesterification reactions	5.37	42.0E-3	Cwc22, Eif4a3, Hnrnpc, Hnrnpl, Lsm7, Lsm8, Magoh, Phf5a, Snrnp70, Snrpd2l, Srsf4
GO:0051351 ~ positive regulation of ligase activity	17.39	42.0E-3	Mid1ip1, Pin1, Stub1, Ube2n

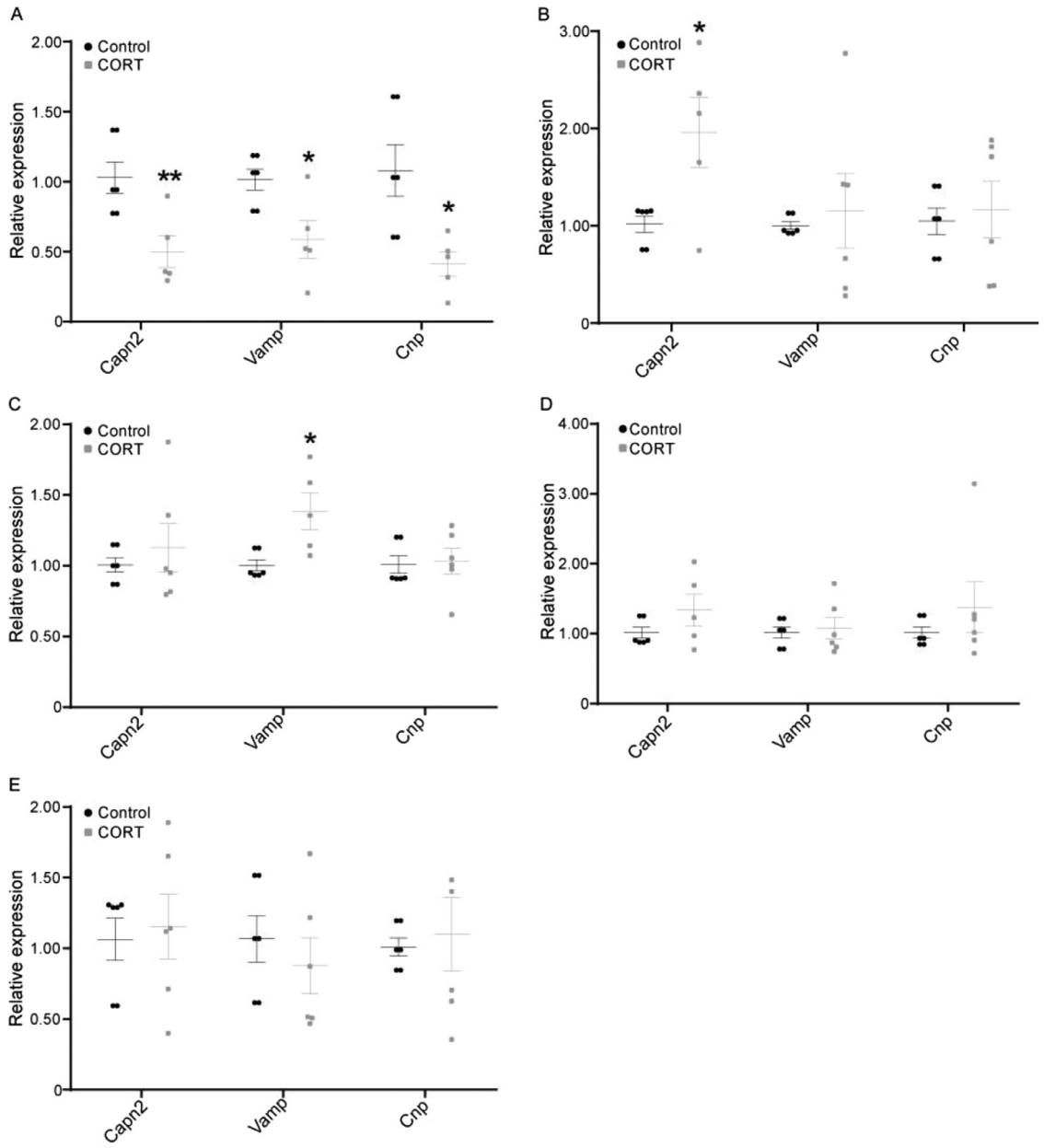

Fig. 3. Chronic corticosterone (CORT) treatments induced changes of gene expression in various brain regions. Changes of gene expression in the HIP (A), PFC (B), RHN (C), Tha (D), and Pit (E), respectively. Data is expressed as the mean ± SEM (n = 6 for each group). *$P \leq 0.05$; **$P \leq 0.01$ ***$P \leq 0.001$ compared with their corresponding Control group. Hip: hippocampus; PFC: prefrontal cortex; RHN: raphe nuclei; Tha: Thalamus; Pit: pituitary.

while it decreased in the hippocampus (Hip) ($P<0.05$). In contrast, *Vamp* increased in the raphe nuclei (RHN) ($P<0.05$), while it decreased in the Hip. Also, *Cnp* decreased in the Hip ($P<0.05$, n = 6) (Fig. 3).

3.6. Chronic unpredicted mild stress (CUMS) changed the expression of Capn2, Vamp and Cnp in various brain regions

Our unpublished data also found that animals showed depressive like behavior after CUMS treatments. Here, we analyzed the effects of CUMS on expression of *Capn2, Vamp* and *Cnp* in the same brain regions as chronic corticosterone treatments. We found increased *Capn2* expression in the PFC ($P<0.01$), RHN, and pituitary (Pit), while decreased expression was noted in both the thalamus (Tha) ($P<0.001$) and the Hip ($P<0.05$). In concordance, *Vamp* expression increased in both the RHN ($P<0.01$) and Pit (P = 0.05). *Cnp* expression increased in the RHN (P = 0.003), Pit, and PFC ($P<0.01$), while it decreased in the Hip ($P<0.05$, n = 6) and in the Tha ($P<0.001$) (Fig. 4).

4. Discussion

The main findings of this paper include: 1) CORT-induced decrease of PC12 cell viability could be reversed by fluoxetine; 2) fluoxetine alleviated neurite growth inhibition induced by CORT in PC12 cells; 3) transcriptome profiling showed that the expression changes of 589 DEGs induced by CORT could be reversed by fluoxetine; 4) genes related to cell morphogenesis, neurite growth, and immune function were involved in the neuroprotective effect of fluoxetine against CORT; 5) expression of neurite growth-related genes, such as *Capn2, Vamp7* and *Cnp*, altered in a brain region- or treatment-specific manner in two different animal models of depression.

Increasing evidence suggested that sustained stress or high level of glucocorticoids adversely affect neuroplasticity, a mechanism

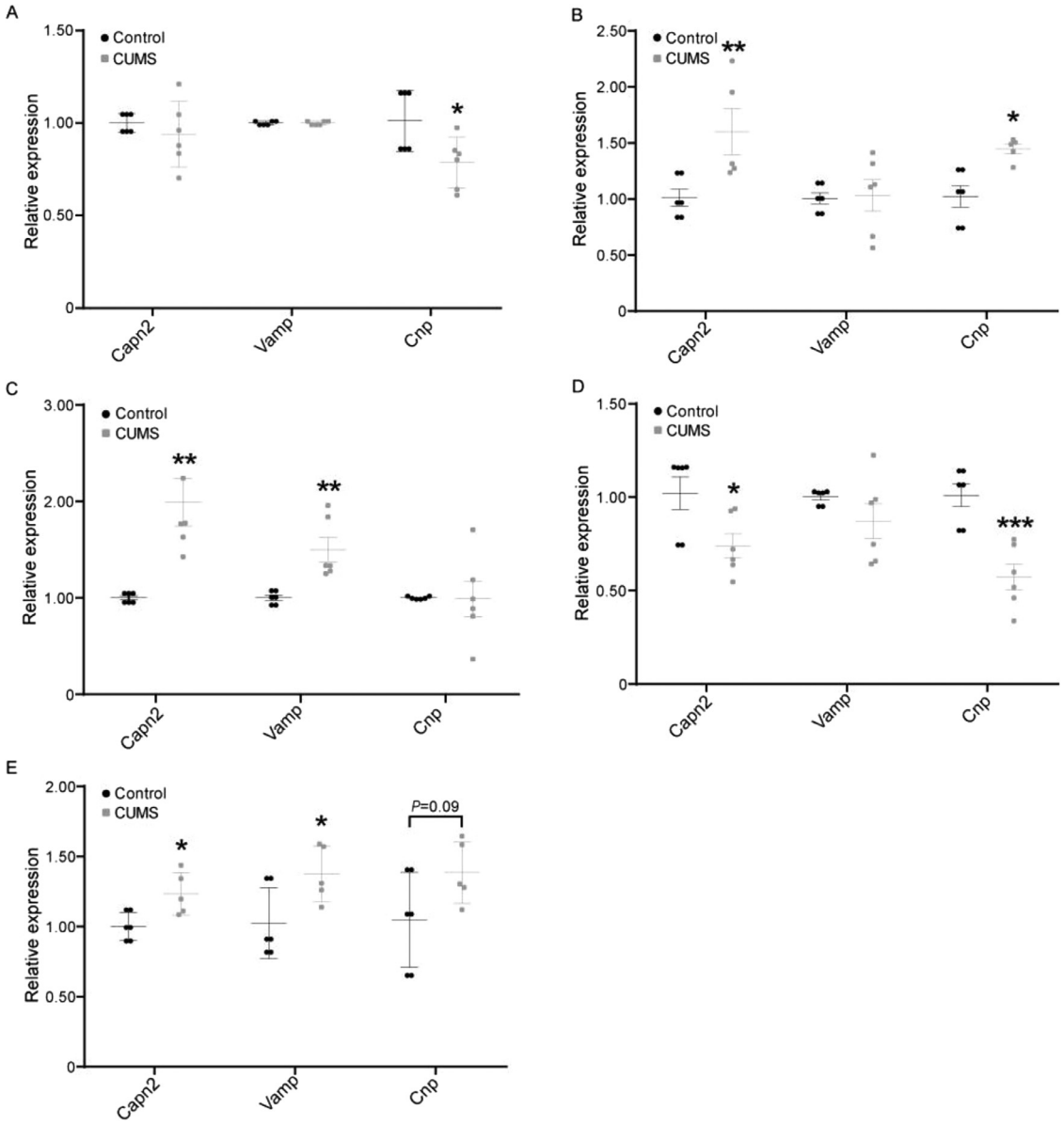

Fig. 4. Chronic unpredicted mild stress (CUMS) treatments induced changes of gene expression in various brain regions. Changes of gene expression in the HIP (A), PFC (B), RHN (C), Tha (D), Pit (E), respectively. Data is expressed as the mean ± SEM (n = 6 for each group). *$P \leq 0.05$; **$P \leq 0.01$ ***$P \leq 0.001$ compared with their corresponding Control group. Hip: hippocampus; PFC: prefrontal cortex; RHN: raphe nuclei; Tha: Thalamus; Pit: pituitary.

fundamental for neuronal adaptation of depression, which could be reversed by antidepressant treatment (Sapolsky, 2000). Specifically, prolonged stress and excessive glucocorticoids have adverse effects at the cellular level, including dendrites processes retraction or neurite outgrowth inhibition (Li et al., 2003; Santarelli et al., 2003). Our results showed that fluoxetine reversed neurite growth inhibition in PC12 cells induced by CORT, which were supported by several previous study. For example, it was reported that dexamethasone could bind to the GR and impair NGF-promoted neurite outgrowth by interfering with the activation/phosphorylation of Akt and ERK1/2 (Terada et al., 2014). Additionally, repeated restraint stress in rats for 3 weeks caused dendrites atrophy of CA3 pyramidal neurons (McEwen et al., 1997). These changes had been involved in a reduction in the amount of neuropil reported in post-mortem studies of the hippocampus of patients with major depression (Sapolsky, 2000).

Furthermore, several signaling pathways were involved in both neuritogenesis and neurite outgrowth. To date, phospholipase C-g (PLC-g), phosphatidylinositol-3 kinase (PI3K), the mammalian target of rapamycin (mTOR), p38 MAPK, c-Jun N-terminal kinase (JNK), Akt, heat shock protein Hsp90a, Wnt-3a, and the Ras/Raf/ERK/MAPK pathways have been identified to play a role in the NGF-induced neurite outgrowth in PC12 cells (Ishima et al., 2012; Selvaraj et al., 2015). In the current study, we identified three new molecular factors, including *Capn2, Vamp* and *Cnp,* which may contribute to neurite outgrowth inhibition induced by CORT.

CALPAIN is an evolutionary conventional family of soluble, neutral, calcium-dependent cysteine proteases, which modify the activity/function of their substrate proteins through protein cleavage (Baudry and Bi, 2016). Calpain has a widespread role in synaptic plasticity via cytoskeletal regulation, AMPA receptor trafficking, actin polymerization and regulation of local protein synthesis (Baudry and Bi, 2016). Recent studies demonstrated that calpain participated in the regulation of dendritic structure by RhoA and p70S6K (Baudry and Bi, 2016). *Capn2* (m-calpain catalytic subunit) belongs to the calpain family and has a well-established causal role in the survival of primary hippocampal neurons following NMDA excitotoxicity (Bevers et al.,

2009).

SYBL1/VAMP7, also referred to as tetanus toxin-insensitive vesicle-associated membrane protein or TI-VAMP, mediates the fusion of intracellular vesicles and the plasma membrane. VAMP7 has an extensive distribution in the adult brain and was implicated in actin remodeling during neurite outgrowth (Alberts et al., 2006; Coco et al., 1999). Furthermore, VAMP7-mediated exocytosis was involved in neuritogenesis through the regulation of membrane delivery coordinated with the actin cytoskeleton (Baudry and Bi, 2016). In addition, loss of VAMP7 reduced AMPA mEPSCs and led to subsequent resting NMDA receptor activation, causing inhibition of synaptic plasticity (Crawford et al., 2017). Furthermore, in vivo evidence showed that VAMP7 contributed to myelin biogenesis by delivering the cargo to the myelin membrane (Feldmann et al., 2011). Importantly, VAMP7 knocked-out male mice showed higher unconditioned basal anxiety level when compared to controls (Danglot et al., 2012).

CNP is an abundant neuropeptide in both the human brain and the cerebrospinal fluid and plays diverse functions in central nervous system. For example, CNP was involved in anxiogenesis, representing a ligand that induces bifurcation of ingrowing sensory axons via its receptor Npr2 (Schmidt et al., 2009). Additionally, CNP caused hyperpolarization of CA3 neurons which increases their input resistance and decreases their inhibitory conductance (Decker et al., 2009). Furthermore, CNP was shown to inhibit brain derived neurotrophic factor and nerve growth factor-induced proliferation of olfactory neuronal precursors and promote differentiation (Simpson et al., 2002). CNP in the sensory nerve was necessary for development and function of dorsal root ganglion (DRG) neurons (Kishimoto et al., 2008).

Together, *Capn2, Vamp7* and *Cnp* participated in neuroplasticity at numerous levels, especially in anxiogenesis, neuritogenesis and neurite growth. Our results showed that both inhibition of neurite growth and changes in the expression of *Capn2, Vamp7* and *Cnp* induced by CORT treatment could be reversed by fluoxetine. To further investigate the potential role of *Capn2, Vamp7* and *Cnp* in depression, we tested the changes of their expression in stress related brain regions in two animal models of depression. Our results showed that all three genes are down-regulated in the hippocampus after chronic CORT treatment, and in the thalamus after CUMS. **They also showed some inconsistence between cell and animal model. Some reasons might be used to explain these differences. Firstly, although PC12 cell line was widely used to investigate the glucocorticoid-induced impairment of neuronal cells, as a cell line derived from a pheochromocytoma of the rat adrenal medulla, it couldn't act like normal physiological neuronal cells. So, it may display different physiological response to the tested challenges. Secondly, the absence of the in vivo biokinetics may lead to a misinterpretation of the in vitro data (Saeidnia et al., 2015). The varying responses among the brain regions induced by CUMS and chronic CORT treatment implying brain-region-specific response in vivo after depression. Of note, *Capn2* showed consistent increase in CORT treated cell line and in PFC of both depressive models, indicating the functional importance of *Capn2* in etiology of depression. However, although PC12 cells and two animal models of depression were used to examine the expression alteration of target genes, functional neurons were also needed in the further study to elucidate the mechanism underlying depression.**

Conclusions: Given the clear relationship among stress, excessive glucocorticoids and major depression, our results suggested that the interaction between stress, glucocorticoids and neurite growth inhibition may be a candidate for the underlying pathophysiology of major depressive disorder (MDD), and that *Capn2, Vamp7* and *Cnp* may represent potential targets for the treatment of MDD.

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Declaration of Interest

The authors have no conflicts of interest to declare.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.psychres.2019.04.017.

References

Alberts, P., Rudge, R., Irinopoulou, T., Danglot, L., Gauthier-Rouviere, C., Galli, T., 2006. Cdc42 and actin control polarized expression of TI-VAMP vesicles to neuronal growth cones and their fusion with the plasma membrane. Mol. Biol. Cell 17 (3), 1194–1203.
Baudry, M., Bi, X., 2016. Calpain-1 and Calpain-2: the Yin and Yang of Synaptic Plasticity and Neurodegeneration. Trends Neurosci. 39 (4), 235–245.
Bevers, M.B., Lawrence, E., Maronski, M., Starr, N., Amesquita, M., Neumar, R.W., 2009. Knockdown of m-calpain increases survival of primary hippocampal neurons following NMDA excitotoxicity. J. Neurochem. 108 (5), 1237–1250.
Blackburn-Munro, G., Blackburn-Munro, R.E., 2001. Chronic pain, chronic stress and depression: coincidence or consequence? J. Neuroendocrinol. 13 (12), 1009–1023.
Chen, G., Yang, D., Yang, Y., Li, J., Cheng, K., Tang, G., Zhang, R., Zhou, J., Li, W., Liu, Z., Fan, S., Xie, P., 2015. Amino acid metabolic dysfunction revealed in the prefrontal cortex of a rat model of depression. Behav. Brain Res. 278, 286–292.
Coco, S., Raposo, G., Martinez, S., Fontaine, J.J., Takamori, S., Zahraoui, A., Jahn, R., Matteoli, M., Louvard, D., Galli, T., 1999. Subcellular localization of tetanus neurotoxin-insensitive vesicle-associated membrane protein (VAMP)/VAMP7 in neuronal cells: evidence for a novel membrane compartment. J. Neurosci. 19 (22), 9803–9812.
Colich, N.L., Kircanski, K., Foland-Ross, L.C., Gotlib, I.H., 2015. HPA-axis reactivity interacts with stage of pubertal development to predict the onset of depression. Psychoneuroendocrinology 55, 94–101.
Crawford, D.C., Ramirez, D.M., Trauterman, B., Monteggia, L.M., Kavalali, E.T., 2017. Selective molecular impairment of spontaneous neurotransmission modulates synaptic efficacy. Nat. Commun. 8, 14436.
Danglot, L., Zylbersztejn, K., Petkovic, M., Gauberti, M., Meziane, H., Combe, R., Champy, M.F., Birling, M.C., Pavlovic, G., Bizot, J.C., Trovero, F., Della Ragione, F., Proux-Gillardeaux, V., Sorg, T., Vivien, D., D'Esposito, M., Galli, T., 2012. Absence of TI-VAMP/Vamp7 leads to increased anxiety in mice. J. Neurosci. 32 (6), 1962–1968.
Decker, J.M., Wojtowicz, A.M., Ul Haq, R., Braunewell, K.H., Heinemann, U., Behrens, C.J., 2009. C-type natriuretic peptide decreases hippocampal network oscillations in adult rats in vitro. Neuroscience 164 (4), 1764–1775.
Dharmawardhane, S., Brownson, D., Lennartz, M., Bokoch, G.M., 1999. Localization of p21-activated kinase 1 (PAK1) to pseudopodia, membrane ruffles, and phagocytic cups in activated human neutrophils. J. Leukoc. Biol. 66 (3), 521–527.
Du, J., Wang, Y., Hunter, R., Wei, Y., Blumenthal, R., Falke, C., Khairova, R., Zhou, R., Yuan, P., Machado-Vieira, R., McEwen, B.S., Manji, H.K., 2009. Dynamic regulation of mitochondrial function by glucocorticoids. Proc. Natl. Acad. Sci. U S A 106 (9), 3543–3548.
Feldmann, A., Amphornrat, J., Schonherr, M., Winterstein, C., Mobius, W., Ruhwedel, T., Danglot, L., Nave, K.A., Galli, T., Bruns, D., Trotter, J., Kramer-Albers, E.M., 2011. Transport of the major myelin proteolipid protein is directed by VAMP3 and VAMP7. J. Neurosci. 31 (15), 5659–5672.
Fujita, K., Lazarovici, P., Guroff, G., 1989. Regulation of the differentiation of PC12 pheochromocytoma cells. Environ. Health Perspect. 80, 127–142.
Gong, S., Miao, Y.L., Jiao, G.Z., Sun, M.J., Li, H., Lin, J., Luo, M.J., Tan, J.H., 2015. Dynamics and correlation of serum cortisol and corticosterone under different physiological or stressful conditions in mice. PLoS One 10 (2), e0117503.
Gourley, S.L., Swanson, A.M., Koleske, A.J., 2013. Corticosteroid-induced neural remodeling predicts behavioral vulnerability and resilience. J. Neurosci. 33 (7), 3107–3112.
Ishima, T., Iyo, M., Hashimoto, K., 2012. Neurite outgrowth mediated by the heat shock protein Hsp90alpha: a novel target for the antipsychotic drug aripiprazole. Transl. Psychiatry 2 (, 10), e170.
Joels, M., Sarabdjitsingh, R.A., Karst, H., 2012. Unraveling the time domains of corticosteroid hormone influences on brain activity: rapid, slow, and chronic modes. Pharmacol. Rev. 64 (4), 901–938.
Kishimoto, I., Tokudome, T., Horio, T., Soeki, T., Chusho, H., Nakao, K., Kangawa, K.,

2008. C-type natriuretic peptide is a Schwann cell-derived factor for development and function of sensory neurones. J. Neuroendocrinol. 20 (11), 1213–1223.
Li, M., Zhou, J., Qian, J., Cheng, X., Wu, H., Li, L., Qian, C., Su, J., Wu, D., Burns, L., Golden, T., Wu, N., 2016. Target genes involved in corticosterone-induced PC12 cell viability and neurite disorders: a potential molecular mechanism of major depressive disorder. Psychiatry Res. 235, 206–208.
Li, Y.F., Liu, Y.Q., Huang, W.C., Luo, Z.P., 2003. Cytoprotective effect is one of common action pathways for antidepressants. Acta Pharmacol. Sin. 24 (10), 996–1000.
Liu, Y., Zhuang, X., Gou, L., Ling, X., Tian, X., Liu, L., Zheng, Y., Zhang, L., Yin, X., 2013. Protective effects of nizofenone administration on the cognitive impairments induced by chronic restraint stress in mice. Pharmacol. Biochem. Behav. 103 (3), 474–480.
Marasco, V., Herzyk, P., Robinson, J., Spencer, K.A., 2016. Pre- and post-natal stress programming: developmental exposure to glucocorticoids causes long-term brain-region specific changes to transcriptome in the precocial Japanese Quail. J. Neuroendocrinol. 28 (5). https://doi.org/10.1111/jne.12387.
Marshall, O.J., 2004. PerlPrimer: cross-platform, graphical primer design for standard, bisulphite and real-time PCR. Bioinformatics 20 (15), 2471–2472.
Martin, M., 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. Marcel Martin, EMBnet.J. , 17, 10–12.
McEwen, B.S., Conrad, C.D., Kuroda, Y., Frankfurt, M., Magarinos, A.M., McKittrick, C., 1997. Prevention of stress-induced morphological and cognitive consequences. Eur. Neuropsychopharmacol. 7 (Suppl 3), S323–S328.
Mpofana, T., Daniels, W.M., Mabandla, M.V., 2016. Exposure to early life stress results in epigenetic changes in neurotrophic factor gene expression in a Parkinsonian Rat Model. Parkinsons Dis. 2016, 6438783.
Pan, Y., Chen, X.Y., Zhang, Q.Y., Kong, L.D., 2014. Microglial NLRP3 inflammasome activation mediates IL-1beta-related inflammation in prefrontal cortex of depressive rats. Brain Behav. Immun. 41, 90–100.
Pollock, J.D., Krempin, M., Rudy, B., 1990. Differential effects of NGF, FGF, EGF, cAMP, and dexamethasone on neurite outgrowth and sodium channel expression in PC12 cells. J. Neurosci. 10 (8), 2626–2637.
Rosa, P.B., Ribeiro, C.M., Bettio, L.E., Colla, A., Lieberknecht, V., Moretti, M., Rodrigues, A.L., 2014. Folic acid prevents depressive-like behavior induced by chronic corticosterone treatment in mice. Pharmacol. Biochem. Behav. 127, 1–6.
Roszkowski, M., Manuella, F., von Ziegler, L., Duran-Pacheco, G., Moreau, J.L., Mansuy, I.M., Bohacek, J., 2016. Rapid stress-induced transcriptomic changes in the brain depend on beta-adrenergic signaling. Neuropharmacology 107, 329–338.
Saeidnia, S., Manayi, A., Abdollahi, M., 2015. From in vitro experiments to in vivo and clinical studies; pros and cons. Curr. Drug Discov. Technol. 12 (4), 218–224.
Santarelli, L., Saxe, M., Gross, C., Surget, A., Battaglia, F., Dulawa, S., Weisstaub, N., Lee, J., Duman, R., Arancio, O., Belzung, C., Hen, R., 2003. Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. Science 301 (5634), 805–809.
Sapolsky, R.M., 2000. Glucocorticoids and hippocampal atrophy in neuropsychiatric disorders. Arch. Gen. Psychiatry 57 (10), 925–935.
Schmidt, H., Stonkute, A., Juttner, R., Koesling, D., Friebe, A., Rathjen, F.G., 2009. C-type natriuretic peptide (CNP) is a bifurcation factor for sensory neurons. Proc. Natl. Acad. Sci. U S A 106 (39), 16847–16852.
Selvaraj, P., Huang, J.S., Chen, A., Skalka, N., Rosin-Arbesfeld, R., Loh, Y.P., 2015. Neurotrophic factor-alpha1 modulates NGF-induced neurite outgrowth through interaction with Wnt-3a and Wnt-5a in PC12 cells and cortical neurons. Mol. Cell. Neurosci. 68, 222–233.
Simpson, P.J., Miller, I., Moon, C., Hanlon, A.L., Liebl, D.J., Ronnett, G.V., 2002. Atrial natriuretic peptide type C induces a cell-cycle switch from proliferation to differentiation in brain-derived neurotrophic factor- or nerve growth factor-primed olfactory receptor neurons. J. Neurosci. 22 (13), 5536–5551.
Terada, K., Kojima, Y., Watanabe, T., Izumo, N., Chiba, K., Karube, Y., 2014. Inhibition of nerve growth factor-induced neurite outgrowth from PC12 cells by dexamethasone: signaling pathways through the glucocorticoid receptor and phosphorylated Akt and ERK1/2. PLoS One 9 (3), e93223.
Tomiyama, A.J., O'Donovan, A., Lin, J., Puterman, E., Lazaro, A., Chan, J., Dhabhar, F.S., Wolkowitz, O., Kirschbaum, C., Blackburn, E., Epel, E., 2012. Does cellular aging relate to patterns of allostasis? An examination of basal and stress reactive HPA axis activity and telomere length. Physiol. Behav. 106 (1), 40–45.
Trapnell, C., Pachter, L., Salzberg, S.L., 2009. TopHat: discovering splice junctions with RNA-Seq. Bioinformatics 25 (9), 1105–1111.
Trapnell, C., Roberts, A., Goff, L., Pertea, G., Kim, D., Kelley, D.R., Pimentel, H., Salzberg, S.L., Rinn, J.L., Pachter, L., 2012. Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. Nat. Protoc. 7 (3), 562–578.
Wang, J., Luo, Y., Wang, K., Wang, Y., Zhang, X., Teng, H., Sun, Z., 2015. Clock-controlled StAR's expression and corticosterone production contribute to the endotoxemia immune response. Chronobiol. Int. 32 (3), 358–367.
Wong, D.L., Tai, T.C., Wong-Faull, D.C., Claycomb, R., Kvetnansky, R., 2008. Adrenergic responses to stress: transcriptional and post-transcriptional changes. Ann. N. Y. Acad. Sci. 1148, 249–256.
Wu, L.M., Hu, M.H., Tong, X.H., Han, H., Shen, N., Jin, R.T., Wang, W., Zhou, G.X., He, G.P., Liu, Y.S., 2012. Chronic unpredictable stress decreases expression of brain-derived neurotrophic factor (BDNF) in mouse ovaries: relationship to oocytes developmental potential. PLoS One 7 (12), e52331.
Zhao, J., Peng, L., Zheng, W., Wang, R., Zhang, L., Yang, J., Chen, H., 2015. Chemically bonding of Amantadine with Gardenamide a enhances the neuroprotective effects against corticosterone-induced insults in PC12 cells. Int. J. Mol. Sci. 16 (9), 22795–22810.
Zou, Y., Wang, B., Fu, W., Zhou, S., Nie, Y., Tian, S., 2016. Apelin-13 protects PC12 cells from corticosterone-induced apoptosis through PI3K and ERKs activation. Neurochem. Res. 41 (7), 1635–1644.