Supporting Information

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SI Materials and Methods

Cell Culture. The immortalized multipotent, human hippocampal progenitor cell line, HPC03A/07 (propriety of ReNeuron), was used for all experiments. As previously described, HPC03A/07 cells were originally obtained from a 12-wk-old male fetus and conditionally immortalized with a transgene encoding for a fusion protein of c-myc and a tamoxifen-inducible estrogen receptor (c-myc-ER^{TM}) (1–3). This construct is exclusively responsive to the synthetic steroid 4-hydroxytamoxifen (4-OHT) (1). HPC03A/07 cells proliferate indefinitely in the presence of epidermal growth factor (EGF), fibroblast growth factor (bFGF) and 4-OHT, and differentiation is induced by removal of EGF, bFGF and 4-OHT (2, 3). During normal expansion, HPC03A/07 cells proliferate with a doubling time of 72 h (4).

HPC03A/07 cells were grown in reduced modified media (RMM) consisting of Dulbecco's Modified Eagle's Media/F12 (DMEM: F12, Invitrogen) supplemented with 0.03% human albumin (Zenalb), 100 μg/mL human apo-transferrin, 16.2 μg/mL human putrescine DiHCl, 5 μg/mL recombinant human insulin, 60 ng/mL progesterone, 2 mM L-glutamine, and 40 ng/mL sodium selenite. To maintain proliferation, 10 ng/mL human bFGF, 20 ng/mL human EGF, and 100 nM 4-OHT were added. The cell culture media is free of any glucocorticoids unless cortisol is added as a treatment in the experimental conditions. To investigate how the presence of albumin influences the effects of cortisol on gene expression, cells were cultured in RMM media without any albumin for a period of 12 h in a subset of experiments.

As described (5, 6), BrdU incorporation for 4 h during proliferation (see below) resulted in 35% BrdU-positive cells in the control condition. Differentiated HPC03A/07 cultures (7 d differentiation) consisted of 35% Dcx-positive neuroblasts, 25% microtubule-associated protein 2 (MAP2)-positive mature neurons, 8% Dcx/MAP2-positive cells and 27% S100β-positive astrocytes.

Proliferation Assay. Progenitor cell proliferation was assessed as described (5, 6). Briefly, HPC03A/07 cells were plated on 96-well plates (Nunclon) at a density of 1.2×10^4 cells per well and cultured for 72 h in the presence of growth factors and 4-OHT to maintain proliferation. The synthetic nucleotide BrdU (10 μM) was added to the media 4 h before treatment cessation. Cells were fixed with 4% paraformaldehyde (PFA) for 20 min at room temperature.

Differentiation Assay. Differentiation was assessed as described (5, 6). Briefly, cells were cultured in the presence of growth factors and 4-OHT for 72 h and subsequently washed and cultured in media without growth factors and 4-OHT for further 7 d to allow differentiation. For these experiments, cells were treated during the initial proliferation phase and the subsequent differentiation phase. At the end of the total incubation time (10 d) cells were fixed as described above.

Drugs. All drugs and reagents were purchased from Sigma-Aldrich unless otherwise stated. Growth factors EGF and bFGF were purchased from Peprotech. GSK650394 was from Tocris Bioscience. Hydrocortisone and RU486 were dissolved in 100% ethanol. GSK650394 was dissolved in 100% DMSO. BrdU was dissolved in PBS fresh before use.

Immunocytochemistry. Neuronal differentiation and maturation was assessed using immunocytochemistry for doublecortin (Dcx) and microtubulin-associated protein-2 (MAP2). Briefly, PFA-fixed cells were incubated in blocking solution [10% normal goat serum (NGS), Alpha Diagnostics] in PBS containing 0.3% Triton-X for 2 h at room temperature, and with primary antibodies (rabbit anti-Dcx, 1:1,000; mouse anti-MAP-2 [HM], 1:500, Abcam) at 4 °C overnight. Specificity of the MAP2 [HM] antibody for mature neurons in our cell culture was previously confirmed (5). Cells were incubated sequentially in blocking solution for 30 min, secondary antibodies (Alexa 594 goat anti-rabbit; 1:1,000; Alexa 488 goat anti-mouse, 1:500, Invitrogen) for 1 h, and Hoechst 33342 dye (0.01 mg/mL, Invitrogen) for 5 min at room temperature. The number of Dcx- and MAP2- positive cells over total Hoechst 33342 positive cells was counted in an unbiased setup with an inverted microscope (IX70, Olympus) and ImageJ 1.41 software (http://rsbweb.nih.gov). To assess progenitor cell proliferation, BrdU-containing cells were incubated with hydrochloric acid (HCl, 2 N) for 15 min at room temperature, blocking solution for 60 min at room temperature, primary antibody (rat anti-BrdU, Serotec, 1:500) at 4 °C overnight, and secondary antibody (Alexa 488 goat anti-rat, 1:500, Invitrogen) for 2 h at room temperature. The number of BrdU-positive cells over total Hoechst 33342-positive cells was determined as described above. Negative controls were incubated with unspecific mouse IgGs (1:500, control for MAP-2), rabbit IgGs (1:500, control for Dcx) or rat IgGs (1:500, control for BrdU) in place of the specific primary antibody.

Gene Expression Analysis. RNA was isolated using RNeasy micro kit (Qiagen) according to the manufacturer's instructions. Samples were treated with DNase (Ambion) and RNA quantity was assessed by evaluation of the A260/280 and A260/230 ratios using a Nanodrop spectrometer (NanoDrop Technologies). RNA quality (RIN) was assessed using Agilent Bioanalyzer (Agilent Technologies). Superscript III enzyme (Invitrogen) was used to reverse-transcribe 1 μg of total RNA. Quantitative Real-Time PCR was performed using HOT FIREPol EvaGreen qPCR Mix (Solis BioDyne) according to the SYBR Green method. PCR cycles consisted of an initial heating step at 95 °C for 15 min to activate the polymerase, 45 PCR cycles were performed. Each cycle consisted of a denaturation step at 95 °C for 30 s, an annealing step at 60 °C for 30 s and an elongation step at 72 °C for 30 s. Primers were designed to amplify 100- to 150-bp regions spanning an exon boundary within the coding sequence of the target gene. GC content of each primer was designed to be 50–55%. For each target primer set, a validation experiment was performed to demonstrate that PCR efficiencies were within the range of 90–100% and approximately equal to the efficiencies of the reference genes. Primer sequences are available upon request.

Each sample was assayed in duplicate and each target gene was normalized to the mean of the three reference genes, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), beta-actin (ACTB); and beta-2-microglobulin (B2M). The Pfaffl Method was used to determine relative target gene expression. Data are expressed as fold change from the vehicle treated control condition.

Affymetrix Gene Expression Microarray and Quality Control. Gene expression microarray assays were performed using Human Genome U219 Array Strips on GeneAtlas platform (Affymetrix), following the 3′IVT one cycle labeling and amplification protocol described in the Affymetrix GeneChip Expression Analysis Technical Manual (http://media.affymetrix.com/support/downloads/manuals/geneatlas_3ivt_expkit_manual.pdf). Human Genome U219 Array Strips are comprised of more than 530,000 probes covering more than 36,000 transcripts and variants, which represent

more than 20,000 genes mapped through UniGene or via RefSeq annotation.

To synthesize first-strand cDNA, 250 ng of RNA were reverse-transcribed with the Gene Atlas 3′IVT Express kit (Affymetrix) using T7 oligo(dT) primer. Second-Strand cDNA synthesis was carried out using DNA polymerase and RNase H to simultaneously degrade RNA and synthesize second-strand cDNA. This step was followed by the in vitro transcription using IVT Labeling Master Mix to generate multiple copies of biotin-modified amplified-RNA (aRNA) from the double-stranded cDNA templates. Subsequently aRNA was purified to remove unincorporated NTPs, salts, enzymes and inorganic phosphate to improve the stability of the biotin-modified aRNA. Labeled aRNA (10 μg) was then fragmented and hybridized onto HGU219 array strips. The reactions of hybridation, fluidics and imaging were performed on the Affymetrix Gene Atlas instrument according to the manufacturer's protocol.

Affymetrix CEL files were imported into Partek Genomics Suite version 6.6 for data visualization and statistical testing. Quality control assessment was performed using Partek Genomic Suite 6.6. All samples passed the criteria for hybridization controls, labeling controls and 3′/5′ Metrics. Background correction was conducted using Robust Multistrip Average (RMA) (7) to remove noise from auto fluorescence. After background correction, normalization was conducted using quantile normalization (8) to normalize the distribution of probe intensities among different microarray chips. Subsequently, a summarization step was conducted using a linear median polish algorithm (9) to integrate probe intensities to compute the expression levels for each gene transcript. Upon data upload, preprocessing of CEL data were performed using ANOVA to assess treatment effects. Differential gene expression across treatment was assessed by applying a P value filter (for treatment) of $P < 0.05$ to the ANOVA results.

Pathway Analysis. Pathway analysis was conducted with Pathway Studio Software 5.0 (Ariadne, Lausanne, Switzerland), using the standard Gene Set enrichment analysis (GSEA), originally developed by the Broad Institute (www.broad.mit.edu/gsea/). This algorithm uses a correlation-weighted Kolmogorov–Smirnov statistic on all gene expression changes and computes pathway enrichment scores by considering gene set membership information, gene list ranking and gene-gene dependencies that reflect real biology. We used $P < 0.005$ as enrichment P value cutoff for pathway analysis.

Nuclear and Cytoplasmic Fractionation. Nuclear extracts were obtained using a Nuclear Extraction kit (Active Motif) according to the manufacturer's instructions. Briefly, cells were washed in ice-cold PBS containing phosphatase inhibitors (Pierce) and calyculin A (Cell Signaling), scraped off the flask and centrifuged for 10 min at 16,000 × g at 4 °C. The supernatant was carefully discarded and cell pellets were gently resuspended in 60 μL of Hypotonic Buffer (Active Motif) containing protease and phosphatase inhibitors and calyculin A. Samples were transferred to microcentrifuge tubes and incubated for 15 min on ice. Detergent (3 μL, Active Motif) was added, and samples were vortexed for 10 s. Samples were centrifuged at 14,000 × g for 2 min at 4 °C, and supernatants (cytoplasmic fractions) were transferred to fresh microcentrifuge tubes and stored at −80 °C. Pellets were resuspended in 30 μL of Complete Lysis Buffer (Active Motif) containing protease inhibitors and calyculin A, vortexed, and incubated for 30 min at 4 °C on a rocking platform at 200 rpm. Lysates were centrifuged for 10 min at 14,000 × g at 4 °C. Supernatants (nuclear fraction) were transferred to fresh microcentrifuge tubes and stored at −80 °C.

Whole Cell Protein Extraction. Cells were washed with ice cold PBS containing phosphatase inhibitors (Pierce) and 0.2 nM calyculin A (Cell Signaling), scraped carefully from the flask and centrifuged in a precooled centrifuge for 10 min at 3,000 rpm at 4 °C. Cell pellets were resuspendend in protein extraction buffer (20 mM Tris·HCl/150 mM NaCl/1 mM EDTA/1 mM EGTA/1% Triton X-100/2 nM calyculin A, protease, and phosphatase inhibitors), and incubated on ice for 15 min. Lysates were centrifuged for 15 min at 14,000 × g at 4 °C. Supernatants were transferred to fresh microcentrifuge tubes and stored at −80 °C.

Western Blot Analysis. Protein concentrations were quantified using a bicinchoninic acid (BCA) colorimetric assay system (Merck). Protein samples containing 50 μg of total protein were boiled for 10 min at 72 °C in 1× NuPAGE LDS sample buffer (Invitrogen) and 1× NuPAGE sample reducing agent (Invitrogen), and subjected to reducing SDS/PAGE on 10% NuPAGE Bis-Tris gels for 1 h at 200 V. Proteins were electrophoretically transferred to Immuno-Blot PVDF membranes (Bio-Rad) at 110 V for 1.5 h at 4 °C. Transfer efficiency was controlled by Ponceau S staining and prestained protein standards. Unspecific binding sites were blocked for 1 h in 5% BSA in TBS containing 0.1% Tween-20 (TBST) and membranes were immunoprobed with the polyclonal rabbit anti-P-S203 (1:10,000) and anti-P-S226 (1:2,000) antibodies (both from Michael J. Garabedian, Department of Microbiology, New York University School of Medicine, New York), anti-P-S211 antibody (1:500; Abcam), anti-GR59 antibody (1:500, Fisher; GR, glucocorticoid receptor), anti–serum- and glucocorticoid-inducible kinase 1 (-SGK1) (1:500, Abcam; SGK1, serum- and glucocorticoid-regulated kinase 1), anti-beta-actin antibody (1:500, Biolegend) and anti-laminB1 antibody (1:500, Abcam) in blocking solution at 4 °C overnight. Membranes were washed with TBST and incubated with Alexa680 goat anti-rabbit secondary antibody (1:10,000, Invitrogen) in 5% nonfat dry milk in TBS for 1 h at room temperature. Membranes were washed in TBST and imaged using the Odyssey Image Station (LI-COR Biosciences). GR protein levels in whole cell lysates and cytoplasmic lysates were normalized to beta-actin (ACTB) protein levels. GR protein levels in the nuclear fraction were normalized to protein levels of the nuclear envelope protein, laminB1.

Recruitment and Assessment of Depressed Patients from the GENDEP Cohort. We investigated SGK1 expression in a subsample of treatment-seeking patients with major depressive disorder from the Genome-based Therapeutic Drugs for Depression (GENDEP) study, which has been extensively described before. Briefly, the GENDEP project is an open-label, part-randomized, multicentre pharmacogenetic study with drug-free depressed patients as well as two active pharmacological treatment arms (10–13). In total, 811 adults with unipolar major depression of at least moderate severity according to both the ICD-10 (14) and the DSM-IV (15) were recruited in eight European countries: Belgium, Croatia, Denmark, Germany, Italy, Poland, Slovenia, and the United Kingdom. To minimize confounding by population stratification, recruitment was restricted to individuals of White European parentage. Personal or family history of bipolar disorder or schizophrenia constituted exclusion criteria. For the current study, we selected 25 patients (10 females and 15 males) who had been drug-free for at least 2 wk before assessment. Patients were on average 36.9 ± 10.7 y old, in their second episode of moderately severe depression, and scored 25.80 (SD 10.5) on the Beck Depression Inventory (BDI) (16), 19.9 (SD 4.1) on the Hamilton Rating Scale for Depression (HRSD) (17), and 25.8 (SD 4.7) on the Montgomery-Asberg Depression Rating Scale (MADRS) (18). Controls (7 males and 7 females) were recruited in London (U.K.) through advertisement in local newspapers, hospitals and job centers, as well as from existing volunteer databases. Controls were screened using the Psychosis Screening Questionnaire (PSQ) (19), and excluded if they met any criteria for a present or past psychotic or mood disorder, or if taking any

kind of hormonal treatment, such as birth-control pills. Controls were on average 36.8 ± 5.8 y old. The study was approved by the Institute of Psychiatry Research Ethical Committee (#292/03) and by the research ethics board of the Free University of Brussels, Belgium, the Central Institute for Mental Health, Mannheim, Germany, the University of Bonn, Germany, the Istituto di Ricovero e Cura a Carattere Scientifico San Giovanni di Dio, Fatebenefratelli Centre, Brescia, Italy, the University of Aarhus, Denmark, the Institute of Public Health, Ljubljana, Slovenia, the University of Medical Sciences, Poznan, Poland, and the University of Zagreb, Croatia. All participants provided written consent after the procedures were fully explained.

Ethics Statement. All animal handling and experimental procedures were performed in accordance with the European Community (European Economic Communities Council Directive 86/609 1987), the Italian legislation on animal experimentation (Decreto Legislativo 116/92), and the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Unpredictable Chronic Mild Stress Procedure. For unpredictable chronic mild stress (UCMS), Sprague–Dawley rats ($n = 8$) were exposed to a variable sequence of mild to moderate, unpredictable stressors once or twice daily for a total duration of 6 wk. Stressors included food or water deprivation, crowding, isolation, soiled caged, 2 h immobilization, light on overnight. Control animals ($n = 8$) were housed under standard conditions. After UCMS, rats were killed by decapitation. The hippocampus was dissected from 2-mm-thick slices corresponding to plates 25–33 (dorsal hippocampus) and plates 34–43 (ventral hippocampus) according to the atlas of Paxinos and Watson (20). Tissue was frozen on dry ice and stored at −80 °C until further analysis.

Prenatal Stress Procedure. Nulliparous adult female (body weight 230–260 g) and male (400 g) Sprague–Dawley rats were pair-housed with a same-sex conspecific with food and water available ad libitum (21 ± 1 °C, 60 ± 10% relative humidity, reversed 12/12 h light/dark cycle). After 10 d, rats were mated for 24 h and individually housed immediately thereafter. Pregnant females were randomly assigned to delivery control (ctrl; $n = 5$) and prenatal stress (PNS; $n = 5$) conditions. PNS consisted of restraining pregnant dams in a transparent Plexiglas cylinder (7.5 cm diameter, 19 cm length) under bright light (6,500 lx) for 45 min three times daily during the last week of gestation. PNS sessions were separated by 2- to 3-h intervals and conducted at varying periods of the day to reduce habituation. Control rats were left undisturbed. Male offspring from control and PNS groups were killed at postnatal day 62 for whole hippocampal dissection.

Statistical Analysis. All statistical analyses were performed with GraphPad Prism 4.03 (GraphPad) on independent biological replicates (indicated as "n"). Data were tested for normality using the Shapiro–Wilk test. Means of two independent treatment groups in nonparametric data sets were analyzed using the Mann–Whitney test. Parametric data were analyzed using one-way ANOVA with Newman–Keuls post hoc test for multiple comparisons among treatment groups. Student t test was used to compare means of two independent treatment groups. Pearson correlation was conducted to correlate gene expression changes. Treatment effects in the gene expression microarray were assessed using Robust MultiChip Average ANOVA. P values < 0.05 were considered significant. Gene Set enrichment analysis for the identification of signaling pathways was conducted using default criteria on the Ariadne Studio software (Mann–Whitney U test as enrichment algorithm, $P < 0.005$ as enrichment P value cutoff). Data are presented as mean ± SEM.

SI Results

Kinases Mediating Cortisol-Induced GR Phosphorylation After 1 h of Treatment. As our data had shown that SGK1 is not involved in phosphorylating the GR serine residues, S203, S211, and S226, upon cortisol treatment for only 1 h, we then sought to determine which kinases are involved in this SGK1-independent effect on initial GR phosphorylation. The cortisol-induced phosphorylation at S203 was counteracted by the CDK2-inhibitor, CVT-313 (1 μM), the CDK5-inhibitor, roscovitine (20 μM), and the ERK-inhibitor, U0126 (20 μM) (Fig. S3*A*); cortisol-induced phosphorylation at the S211 site was counteracted by CVT-313 (1 μM) and, to a lesser extent, by U0126 (20 μM) (Fig. S3*B*); phosphorylation at S226 was not affected by any of the inhibitors used (Fig. S3*C*), suggesting the involvement of kinases not yet known to be implicated in S226 phosphorylation in human hippocampal progenitor cells.

Effects of Cortisol Are Limited by Binding to Albumin. We had previously shown that high concentrations of cortisol (10–100 μM) are required to significantly induce GR transactivation and to elicit predominant GR- over MR-mediated effects of cortisol on progenitor proliferation in this cellular model (6). We hypothesized that such high concentrations of cortisol are required because much of the cortisol may be bound to human albumin in our in vitro model. Therefore, we tested whether the presence of albumin in the cell culture media of HPC03A/07 cells has indeed functional consequences for the effects of cortisol. We treated cells with cortisol (1 μM, 100 μM) for 12 h in the presence albumin (that is, under the same conditions as all our in vitro experiments) and in the absence of any albumin (that is, under conditions in which no cortisol can be bound to albumin). We then investigated cortisol-induced mRNA expression of the GR target genes, *FK506 binding protein 5* (*FKBP5*) and *SGK1*, in both conditions. Indeed, cortisol treatment in the absence of any albumin induced a greater increase in both, *FKBP5* and *SGK1* gene expression compared with cortisol treatment in the presence of albumin (Fig. S6 *A* and *B*). These data thus indicate that the presence of human albumin in the HPC03A/07 cell culture media indeed limits the bioactive availability of cortisol in our in vitro experiments.

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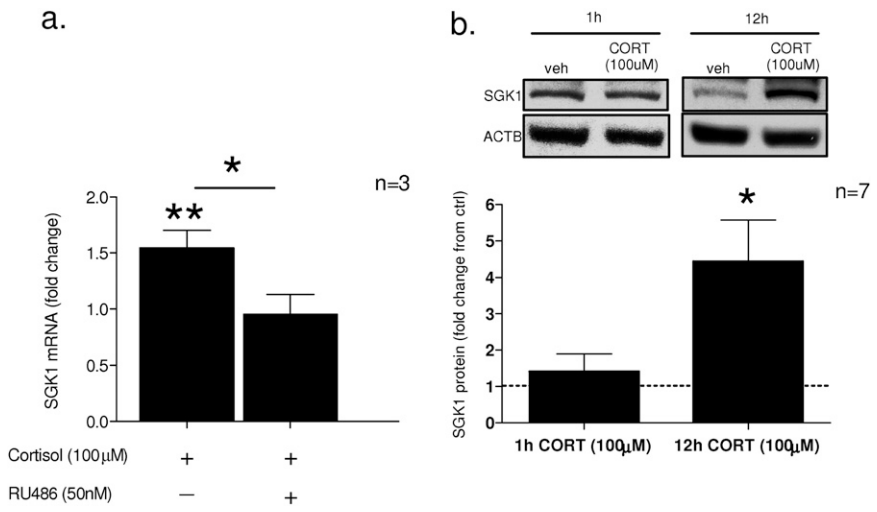

Fig. S1. Cortisol (100 μM) increases SGK1 mRNA and protein expression. (*A*) The cortisol-induced increase in SGK1 mRNA expression after 12 h is counteracted by cotreatment with RU486 (50 nM). $n = 3$. (*B*) Cortisol increases SGK1 protein expression after 12 h of treatment. $n = 7$. Data are mean ± SEM $^{*}P < 0.05$, $^{**}P < 0.01$; compared with the vehicle-treated control condition or as indicated.

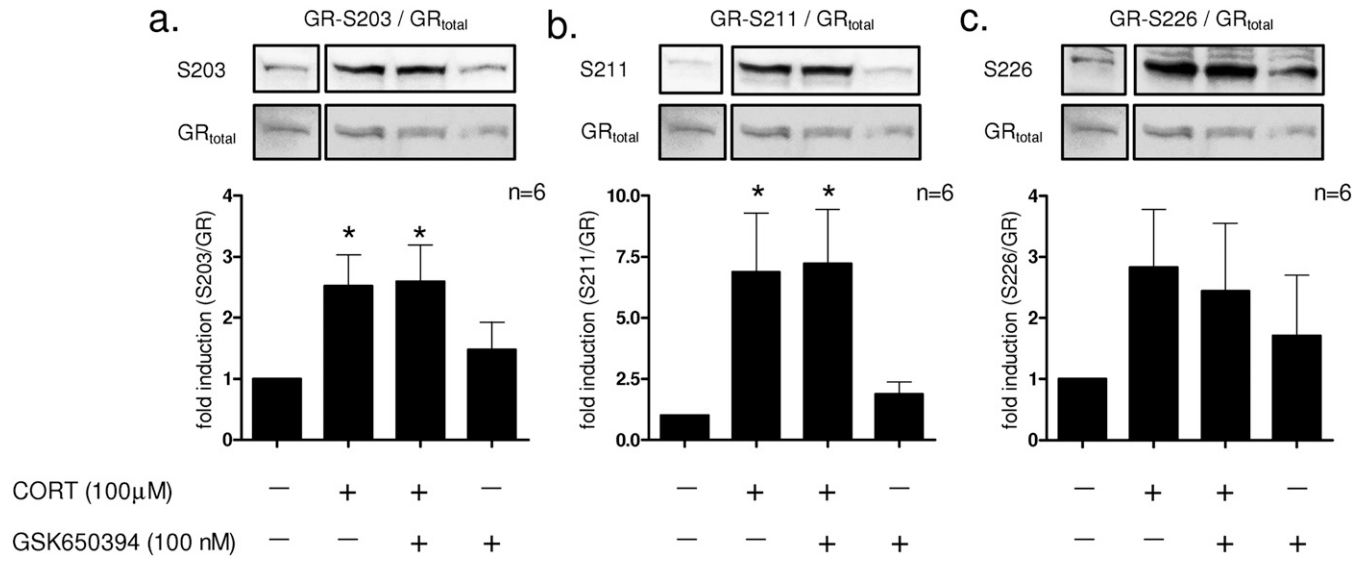

Fig. S2. SGK1 does not mediate cortisol effects on GR phosphorylation after 1 h of treatment. Cortisol (100 μM) increases GR phosphorylation at S203 (*A*), S211 (*B*), and S226 (*B*). These effects were not counteracted by GSK650394 (100 nM) after 1 h of cotreatment. Data are mean ± SEM; $^{*}P < 0.05$ compared with the vehicle-treated control condition. $n = 6$.

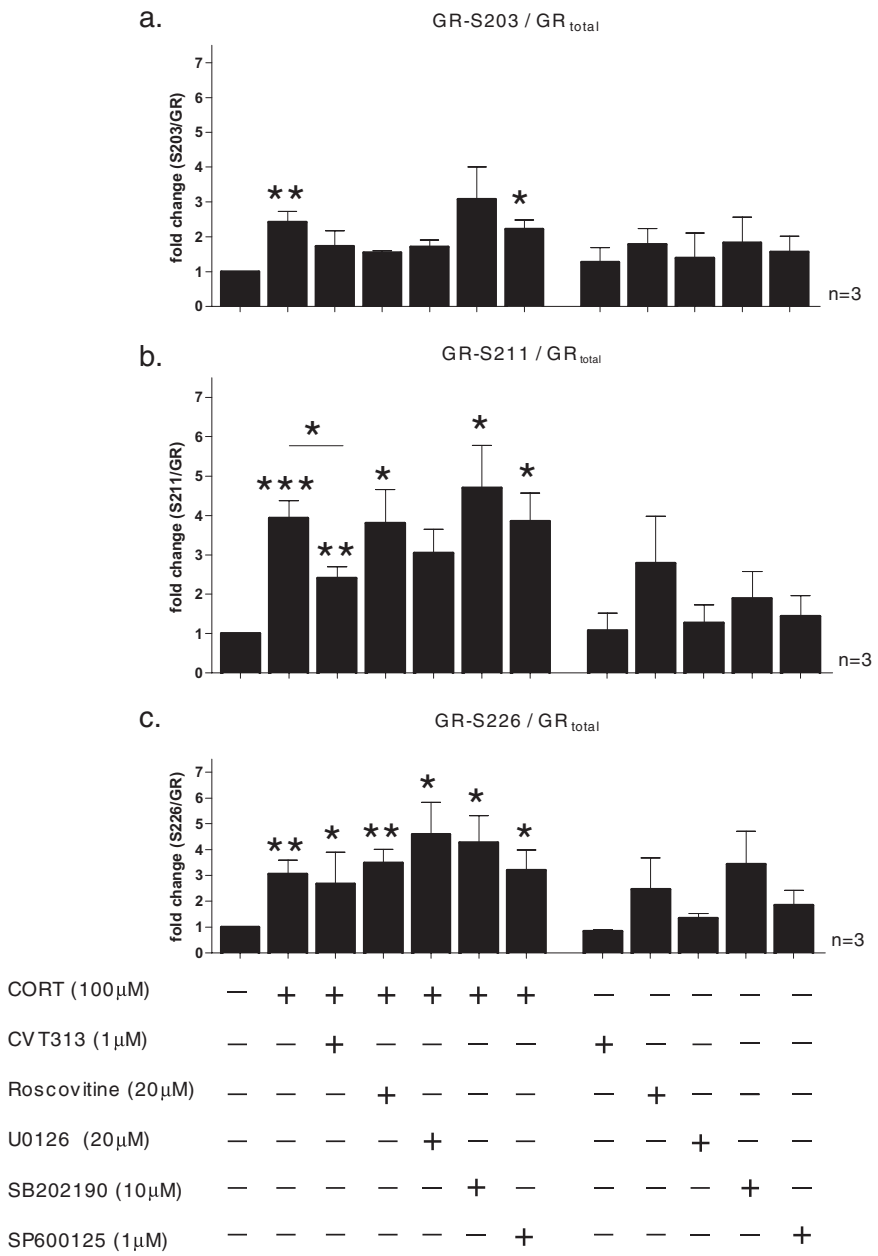

Fig. S3. Kinases involved in GR phosphorylation after 1 h of cortisol treatment. (*A*) The cortisol (100 μM)-induced increase in GR phosphorylation at the S203 site was reduced by CVT313 (1 μM), roscovitine (20 μM), and U0126 (20 μM). (*B*) No effect was observed after cotreatment with SB202190 (10 μM), or SP600125 (1 μM). Phosphorylation at the S211 site was reduced by CVT313. (*C*) No significant changes in GR phosphorylation were observed at the S226 site for any of the kinase inhibitors tested. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ compared with the vehicle treated control condition or as indicated. $n = 3$.

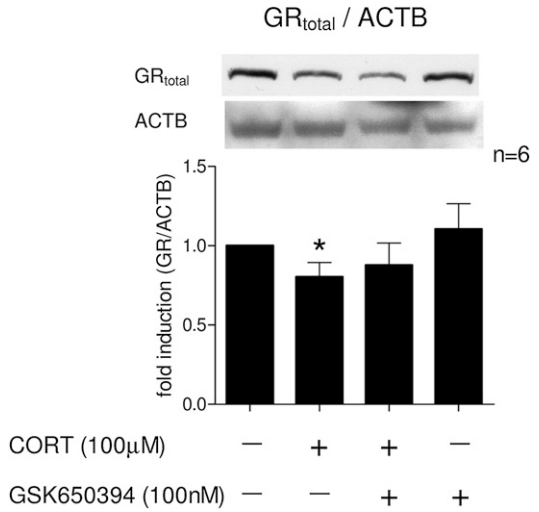

Fig. S4. Cortisol (100 μM) decreases GR protein expression in whole cell lysates after 12 h of treatment. This effect is not counteracted by cotreatment with GSK650394 (100 nM). Data are mean ± SEM *$P < 0.05$ compared with the vehicle treated control condition. $n = 6$.

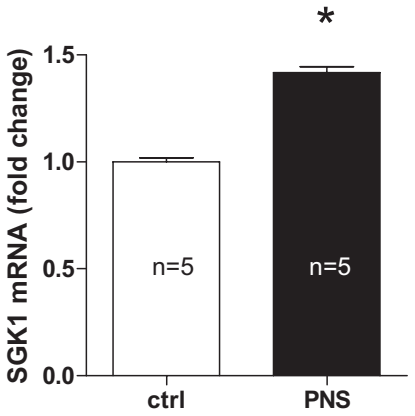

Fig. S5. SGK1 mRNA expression is increased in whole hippocampal lysates of rats at postnatal day 62 after prenatal stress (PNS, $n = 5$). *$P < 0.05$ compared with control.

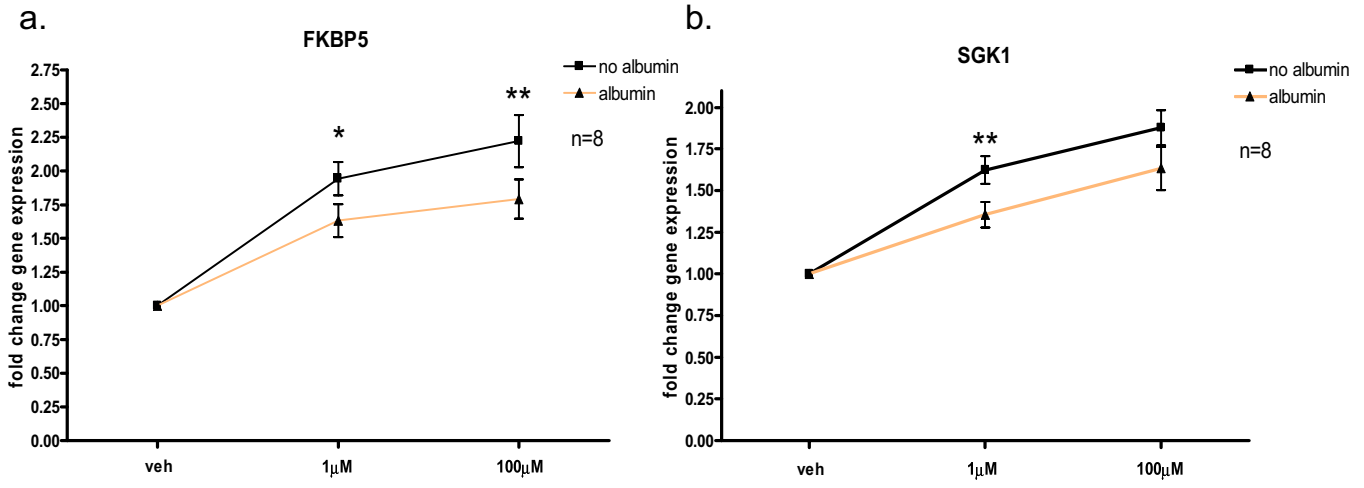

Fig. S6. The effects of cortisol are reduced by the presence of albumin. Treatment with cortisol (1 μM, 100 μM) for 12 h elicits stronger effects on mRNA expression of the GR target genes *FKBP5* (*A*) and *SGK1* (*B*) in the absence of human albumin (black line), whereas the presence of albumin in the cell culture media reduces the effects of cortisol (orange line). *$P < 0.05$, **$P < 0.001$ for the comparison of effect between cortisol treatment with and without albumin. $n = 8$.