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Expression Profiles of the Nuclear Receptors and Their Transcriptional Coregulators During Differentiation of Neural Stem Cells

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Abstract

Neural stem cells (NSCs) are pluripotent precursors with the ability to proliferate and differentiate into 3 neural cell lineages, neurons, astrocytes and oligodendrocytes. Elucidation of the mechanisms underlying these biologic processes is essential for understanding both physiologic and pathologic neural development and regeneration after injury. Nuclear hormone receptors (NRs) and their transcriptional coregulators also play crucial roles in neural development, functions and fate. To identify key NRs and their transcriptional regulators in NSC differentiation, we examined mRNA expression of 49 NRs and many of their coregulators during differentiation (0–5 days) of mouse embryonic NSCs induced by withdrawal of fibroblast growth factor-2 (FGF2). 37 out of 49 NRs were expressed in NSCs before induction of differentiation, while receptors known to play major roles in neural development, such as THR, RXRs, RORs, TRs, and COUPTFs, were highly expressed. CAR, which plays important roles in xenobiotic metabolism, was also highly expressed. FGF2 withdrawal induced mRNA expression of ROR, RXR, and MR by over 20-fold. Most of the transcriptional coregulators examined were expressed basally and throughout differentiation without major changes, while FGF2 withdrawal strongly induced mRNA expression of several histone deacetylases (HDACs), including HDAC11. Dexamethasone and aldosterone, respectively a synthetic glucocorticoid and natural mineralocorticoid, increased NSC numbers and induced differentiation into neurons and astrocytes. These results indicate that the NRs and their coregulators are present and/or change their expression during NSC differentiation, suggesting that they may influence development of the central nervous system in the absence or presence of their ligands.

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Conflict of Interest

The authors do not have any conflict of interest.

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Keywords

central nervous system; histone deacetylase; glucocorticoid; mineralocorticoid; neuron

Introduction

Nuclear hormone receptors (NRs) are intracellular molecules that mediate the actions of their endogenous lipophilic ligands in a broad spectrum of physiological processes [1]. NRs strongly influence the structure and functions of the central nervous system (CNS) by affecting development, growth, differentiation, apoptosis, and death of its component cells. For example, thyroid hormones and their receptors are essential for normal development and function of the CNS, while steroid hormones, such as glucocorticoids, mineralocorticoids, androgens, and estrogens, secreted from the adrenal glands and/or gonadal organs, affect mood, cognitive function and sexual differentiation through their respective receptors expressed in CNS [2 – 4].

Humans and mice respectively express 48 or 49 NRs, and they exert their diverse actions by acting as ligand-dependent transcription factors [1]. NRs efficiently stimulate or repress the transcriptional activity of their target genes either by binding to their cognate DNA sequences (called response elements) located in the regulatory regions of their responsive genes or by interacting with other transcription factors [5]. In the nucleus, DNA- or transcription factor-associated NRs attract their transcriptional coregulators and chromatin-associated molecules [6]. These cofactors influence RNA polymerase II and components of the general transcription machinery, alter the chromatin structure through chemical modification of histones, and ultimately stimulate or repress the transcriptional activity of NR target genes [6]. Currently, over 200 NR cofactor molecules have been reported: These include histone acetyl-transferase coactivators, such as the CREB-binding protein (CBP), p300 and nuclear receptor coactivators (NCoAs), nuclear receptor corepressors (NCoRs), histone deacetylases (HDACs), histone methyltransferases, and further, RNA coregulators, such as the steroid receptor RNA coactivator (SRA) and the growth-specific arrest 5 (Gas5) [6, 7].

The CNS contains populations of neural stem cells (NSCs), defined by their properties of self-renewal and ability to differentiate into multiple neural cell lineages, such as neurons, astrocytes and oligodendrocytes [8]. During development, NSCs provide the cells that will comprise the CNS. In the adult CNS, populations of NSCs still persist, and their localization and biological roles are intensely studied: 2 areas of the adult brain, the dentate gyrus of the hippocampus and the subventricular zone lining the lateral ventricles, are the most established neurogenic zones containing NSCs, while other brain areas comprising NSCs are increasingly being reported [9]. Interestingly, NSCs share some characteristics with the putative progenitor cells found in the adult adrenal medulla, suggesting that NSCs or their related cells may also present in this peripheral neuronal tissue [10, 11]. The roles of adult NSCs may include the supply of new neurons to the olfactory bulb (from the subventricular zone NSC population) and to the hippocampus, thus their self-renewal and differentiation into neural cell lineages potentially affect olfactory function and memory consolidation subserved by these brain areas. More recently, neuroprotective roles of adult NSCs were reported, as NSCs demonstrated powerful neuronal rescue in models of neurodegenerative diseases and neurologic damage induced by pharmacologic treatments [9, 12 – 14].

These pieces of evidence indicate that elucidation of the mechanisms that influence self-renewal and differentiation of NSCs is essential for both understanding the physiology of CNS development and establishing rational therapeutic approaches for neurodegenerative

disorders and CNS injury. Recently, primary cell cultures of NSCs have been established, showing functions reminiscent of their in vivo biology [9, 12, 13]; such systems appear useful in testing pertinent hypotheses and rescuing in vivo brain functions [13]. In these in vitro NSCs, maintenance of their characteristics as stem cells, such as self-renewal and differentiation into 3 neural lineages, is absolutely dependent on the presence of fibroblast growth factor-2 (FGF2) in the culture media [13]. Thus, we employed this valuable cellular system – the mouse primary NSCs – and examined mRNA expression of 49 NRs and 35 selected coregulators before and in the course of differentiation induced by withdrawal of FGF2 to identify key NRs and their transcriptional coregulators in the steady state and differentiation process of NSCs. We found that some NRs and coregulators show distinct expression profiles and dramatically altered expression levels during differentiation. Activation of such NRs may offer targets for NSC proliferation and differentiation into neural cells.

Materials and Methods

Preparation of mouse NSCs and treatments

Mouse NSCs were prepared from mouse embryos at embryonic day 13.5, as reported previously [8, 12]. Briefly, brains were obtained from mouse fetus at E13.5 day, and their cortex was harvested, triturated by pipetting, and cultured in N2 medium (Dulbecco's modified Eagle's medium/F-12 supplemented with 0.1 mg/ml of apo-transferin, 25 µg/ml of insulin, 0.1 µM of putrescine, 30 nM of selenite, 2×10^{-8} M of progesterone, 10 U/ml of penicillin, 100 µg/ml of streptomycin and 0.25 µg/ml of amphotericin B) in the presence of 30 ng/ml of FGF2 for 4–5 days to isolate mouse NSCs. The purified NSCs were then maintained in N2 medium supplemented with 30 ng/ml of FGF2 daily. NSCs were plated in 10 cm dishes at the concentration of 10^5 cells/plate. One day after plating, medium was changed to N2 medium without FGF2, and cells were further cultured for another 5 days. During the culture, total RNA was purified daily (Fig. 1). NSCs were also plated in 24-well plates, cultured in the presence or absence of the steroids indicated, the HDAC inhibitor trichostatin- A (TSA), and FGF2 for 2 days, and cell numbers were then counted.

PCR arrays

The custom PCR arrays, which have primer pairs for 49 mouse NRs/orphan receptors/membrane receptors, 35 coactivators/corepressors/chromatin modulators and 5 control genes (total 89 genes) listed in Table 1, were produced by SABiosciences Corp. (Frederick, MD), as described previously [15]. Total RNA was purified from mouse NSCs by using the RNeasy Mini kit (Qiagen Inc., Valencia, CA, USA), and was treated with DNase (Promega). The samples were further reverse transcribed into cDNA by using the RT² First Strand Kit (SABiosciences Corp.), and real-time PCR reactions were run on the 7 500 Real-time PCR System (Applied Biosystems, Foster City, CA, USA), by following company's instructions.

SYBR Green-based real-time PCR

mRNA expression of some NRs and coregulators that demonstrated characteristic expression patterns was further confirmed by using regular SYBR Green-based real-time PCR. Total RNA samples obtained from mouse NSCs were reverse-transcribed to cDNA with the TaqMan reverse transcription reagents (Applied Biosystems), and real-time PCR was performed in triplicate using the SYBR Green PCR Master Mix (Applied Biosystems) in the 7500 Real-time PCR System (Applied Biosystems), as described previously [16]. The primer pairs used for measuring mRNA levels of THR, ROR, COUP-TFI, ERR, GR, MR, AR, PR, LRH1, PMC11, and HDAC11 in this assay are shown in Table 1. Obtained threshold cycle (C_t) values of these molecules were normalized for those of the acidic ribosomal phosphoprotein P0 (RPLP0) and their relative mRNA expressions are

demonstrated as fold induction over baseline. The dissociation curves of the primer pairs used showed a single peak and samples after PCR reactions had a single expected DNA band in agarose gel analysis (data not shown).

Indirect immunostaining

Mouse NSCs were plated on 24-well plates or 2-well slide chambers, and were incubated with the indicated steroid(s) or TSA in the absence of FGF2. Cells were then fixed with 4 % paraformaldehyde and the expression of the glial fibrillary acidic protein (GFAP: astrocyte marker) and neuron-specific class III β -tubulin (TUJ1: neuron marker) was examined by staining with their specific antibodies (purchased from Dako North America, Inc., Carpinteria, CA, USA and R & D Systems, Minneapolis, MN, USA, respectively) followed by appropriate fluorescence-conjugated secondary antibodies. Cells were also stained with the 4',6-diamidino-2-phenylindole (DAPI). Some cells were cultured in the absence of FGF2 for 5 days, and they were stained with anti-MR (H-300, catalogue # sc-11412, lot # D1709) or -HDAC11 antibody (Q-5, catalogue # sc-101065) purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Staining of these molecules was evaluated under the Leica DMIRB inverted microscope (Leica Microsystems Inc., Bannockburn, IL, USA). Nomarski and fluorescence images were obtained with the C4742-95 CCD camera (Hamamatsu Photonics, Bridgewater, NJ, USA), and were analyzed with the OpenLab v2.2.5 software (Agilent Technologies Inc., Santa Clara, CA, USA).

Statistical analysis

Statistical analyses were carried out by unpaired Student's *t*-test with the 2-tailed p-value.

Results

Many NRs and all coregulators examined are expressed in NSCs

We first examined mRNA expression of 49 NRs and some coregulators in mouse NSCs maintained in the presence of FGF2. *C_t* values of these molecules are shown in Table 2. Thirty seven out of 49 NRs and all (35) coregulators examined were expressed in these cells based on the criterion that the *C_t* value ≤ 35 was the lowest limit for expression. The mean *C_t* value of expressed NRs was 28.62 ± 0.74 , while that of coregulators was 26.13 ± 0.74 ($p = 0.025$), indicating that coregulators tend to be expressed at higher levels than NRs (Fig. 2a).

mRNA of the NRs known to have a significant impact on CNS development and function, such as the thyroid hormone receptor (THR), retinoid X receptors (RXRs), and chicken ovalbumin upstream promoter-transcription factors (COUP-TFs), NUR77 and v-Erba related 2 (EAR2) [2, 17 – 19], were highly expressed in NSCs. In addition to these receptors, the peroxisome proliferator-activated receptor α , testicular receptor (TR) 2 and 4, and the constitutive androstane receptor (CAR), which play important roles in fatty acid, retinoid and xenobiotic metabolism [20 – 22], were abundantly expressed. Among steroid hormone receptors, mRNA of the glucocorticoid (GR), androgen (AR) and progesterone receptor (PR) were moderately expressed in NSCs, while the estrogen receptor (ER) α , ER and mineralocorticoid receptor (MR) were poorly expressed or undetectable. Other unexpressed NRs were the retinoic acid receptor (RAR) α and β , PPAR α , farnesoid X receptors (FXRs), vitamin D receptor (VDR), hepatocyte nuclear receptor 4 (HNF4 α), estrogen-related receptor (ERR) α , neuron-derived orphan receptor 1 (NOR1), and small heterodimer partner (SHP). Among the membrane-associated receptors, the progesterone membrane component 11 (PMC11) was moderately expressed. For coregulators, mRNA of CBP and p300, NCoAs, thyroid hormone receptor-associated protein (TRAP) 220 and 150, HDAC1, 3, 6, and 7, NCoRs, Sin3A, Set/temperature-activating factor (TAF)-I α ,

coactivator-associated arginine methyltransferase 1 (CARM1), HRMT1-like 2, C-terminal tail-binding protein 1 (CtBP1), SNF2 histone linker PHD RING helicase (SHPRH) and the SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily A member 4 (SMARCA4) were all highly expressed in NSCs.

Alteration of NR and coregulator mRNA expression upon differentiation of NSCs

We next examined mRNA expression profiles of NRs and coregulators during differentiation of NSCs by culturing them in the absence of FGF2 for up to 5 days (Fig. 2b, c and Suppl. info Table 1). Both fold increase and decrease of NRs after differentiation were greater than changes of coregulator expression ($p = 0.007$ and 0.001 , respectively), indicating that FGF2 withdrawal and subsequent differentiation of NSCs affected more significantly the NR mRNA expression than that of coregulators.

These results further indicate that NR-mediated biological changes observed during differentiation may be regulated more significantly at the levels of NRs than at those of their coregulators. Among NRs, THR (mean fold expression \pm S.E.: 32.98 ± 30.68 on the 2nd day after FGF2 withdrawal), ROR (40.68 ± 8.28 , 4th day), RXR (50.94 ± 16.77 , 2nd day) and MR (32.73 ± 15.80 , 4th day) showed the most significant expression changes, while COUP-TFI, ERR and LHR1, which play important roles in the development of CNS [2, 18, 23, 24], demonstrated moderate changes (2.5–5-fold changes).

We confirmed mRNA expression of some of these NRs using SYBR Green-based real-time PCR (Fig. 3a, c). Since MR demonstrated a quite dramatic change, we examined its mRNA expression together with that of other steroid hormone receptors in this assay system (Fig. 3b). Significant expression of MR mRNA (over 20-fold) upon differentiation was observed, while GR mRNA expression was elevated by 2-fold. mRNA expression of PR was reduced by ~80 %, whereas that of AR showed minor fluctuations. The progesterone membrane receptor component 11 (PMC11), a membrane steroid hormone receptor expressed in NSCs in the presence of FGF2, did not show any detectable changes in its mRNA expression in the SYBR Green-based realtime PCR (Fig. 3c). Among coregulators, HDAC2, HDAC11, HDAC4 and HDAC10 demonstrated significant mRNA increase (13.23 ± 2.73 , 2nd day; 11.35 ± 2.72 , 4th day; 8.79 ± 2.70 , 2nd day and 6.76 ± 1.87 , 2nd day, respectively) in the screening using the PCR arrays, while mRNA expression of HDCA11 demonstrated an ~ 8-fold increase in the SYBR Green-based real-time PCR (Fig. 3c). mRNA expression of p300 (4.66 ± 2.54 , 2nd day), nuclear receptor-interacting protein 1 (4.06 ± 1.06 , 2nd day), nuclear receptor coregulator 6 (5.04 ± 2.43 , 2nd day), nuclear receptor corepressor 1 (5.83 ± 2.59 , 2nd day) and SRA (6.92 ± 4.79 , 2nd day) demonstrated moderate increases upon differentiation. By using indirect immunostaining, we confirmed that MR and HDCA11 were also increased at the protein level upon differentiation (Fig. 3d).

Glucocorticoid, mineralocorticoid and TSA alter the numbers of NSCs

MR was one of the most highly induced NRs upon NSC differentiation. This receptor, together with the classic receptor GR, mediates strong and diverse actions of glucocorticoids in the CNS, as the MR has a higher affinity to glucocorticoids than GR and the CNS does not express 11 β -hydroxysteroid dehydrogenase type 2 (HSD2), the enzyme that converts active corticosterone into inactive 11-dehydrocorticosterone [5, 25]. Thus, we focused on these receptors, and examined the effects of the synthetic glucocorticoid dexamethasone and its receptor inhibitor RU 486, and the endogenous mineralocorticoid aldosterone and its inhibitor spironolactone on cell numbers of NSCs by culturing them for 2 days in the presence of the steroid(s) and FGF2. We also tested the general HDAC inhibitor TSA in the same assay system, as mRNA expression of several HDACs was highly regulated upon differentiation. Fig. 4a shows images of DAPI staining of cells cultured with the indicated

compounds, while Fig. 4b demonstrates percentage changes of the cell numbers before and after culturing with these compounds. Both dexamethasone and aldosterone significantly increased numbers of NSCs, with the former showing a more pronounced effect. Since these effects were suppressed by co-incubation with their receptor antagonists, we suggest that the observed effects were mediated by the GR and MR, respectively. TSA treatment reduced NSC numbers by ~ 50 % after 2-day culture. Since we did not observe massive cell death during the culture with FGF2, these compounds and/or TSA, it appears that dexamethasone and aldosterone stimulated proliferation of NSCs, while TSA suppressed it. This further suggests that HDACs are necessary for NSCs to maintain their potential for proliferation/self-renewal.

Dexamethasone and aldosterone, but not TSA, alter differentiation of NSCs into neurons and astrocytes

Since GR, MR and HDACs have stimulatory effects on NSC numbers, we next examined dexamethasone, aldosterone, their antagonists and TSA on the differentiation of NSCs into the glial and neuronal lineages, by culturing these cells for 2 days in the presence of these steroid(s) and/or TSA during the differentiation process. We subsequently stained them with the antibodies for GFAP (astrocytes: green) and TUJ1 (neuron: red) for identifying the corresponding neural cells. Given the strong effects of these compounds within the brief treatment period in our experimental setup and slow differentiation of oligodendrocytes and the late expression of their specific cell markers after FGF2 withdrawal, we limited our studies to the glial and neuronal fates, omitting analysis on oligodendrocytes. Fig. 5a demonstrated Nomarski images of the cells treated with the indicated compounds. Dexamethasone significantly increased astrocytes and neurons, while addition of RU 486 strongly suppressed differentiation of NSCs into these cells. Aldosterone increased neuron numbers but not those of astrocytes. TSA had no effect on the differentiation of NSCs (Fig. 5b, c). Percentages of the neurons and astrocytes differentiated from NSCs and those remaining undifferentiated are shown in Table 3.

Discussion

We showed that many NRs and their coregulators were expressed in NSCs and their expression was altered during differentiation induced by FGF2 withdrawal. These findings indicated that the NR signaling pathways are an integral component of NSC self-renewal and differentiation into 3 neural cell lineages, possibly by mediating the biologic actions of their ligands, such as steroids, lipid metabolites and other compounds available in the environment or produced during cell culturing. Importantly, mRNA expression of many NRs and coregulators were highly elevated or reduced at specific time points after FGF2 withdrawal, suggesting that their effects on NSC differentiation may have specific windows during the differentiation process of these cells.

As expected, THR, RXRs, COUP-TFs, NUR77 and EAR2, which are known to play important roles in the development/function of the CNS during the fetal life and/or after birth, were highly expressed in NSCs, and their expression levels were relatively constant throughout the differentiation process, consistent with their general and important roles in CNS biology. We also found that PPAR, TR2 and 4, and CAR were constitutively expressed in NSCs at high levels. Indeed, PPAR ligands are known to stimulate differentiation of NSCs into oligodendrocytes and to suppress inflammation induced by radiation in microglia [20, 26 – 28]. TR2 and 4, the orphan receptors known to play important roles in spermatogenesis and lipid and lipoprotein metabolism [21], are expressed in the embryonic brain particularly in highly proliferating regions, such as the subventricular zone where embryonic NSCs are accumulated, and are required for normal CNS development [21]. Recent reports indicate that TR2 accumulates in the plaques in the brains

of patients with Alzheimer's disease, while TR4 might function as a receptor for vitamin A [29, 30]. CAR plays a central role in the xenobiotic response by binding to many exogenous compounds like phenobarbital, and by inducing the enzymes for catalyzing their clearance from the body [29]. It would, thus, be quite interesting to examine in future the impact of xenobiotic compounds on NSC activities and subsequent CNS development.

We found that mRNA and protein expression of the MR were highly regulated upon NSC differentiation. In the brain, MR mediates the actions of glucocorticoids in addition to those of mineralocorticoids [31]: These hormones are 2 major steroids produced and secreted from the adrenal glands, and respectively play central roles in the adaptive response to stress by functioning as end products of the hypothalamic-pituitary-adrenal (HPA) axis, and electrolytes and water absorption in the kidney by regulating sodium-potassium exchange in the cortical collecting tubules of this organ [5, 32]. Since corticosterone circulates at 2 log units higher concentrations than the natural mineralocorticoid aldosterone and MR has higher affinity to corticosterone than GR [33], basal physiologic levels of circulating corticosterone fully activate the MR in the CNS, which does not express 11 HSD2, while GR is affected primarily by elevated levels of corticosterone during the circadian surge or during stress [31]. NSCs are found in the dentate gyrus of the hippocampus in humans throughout the lifespan and produce granular cell neurons, which support consolidation of new memories [34]. Interestingly, this portion of the brain expresses high levels of GR and MR, and plays important roles in learning, memory consolidation, emotion and in the control of the HPA axis [31]. Further, increasing cortisol response as occurs in stress is positively associated with encoding of new memories in young subjects, while it correlates negatively with efficiency of their retrieval in older subjects [35]. We found that both dexamethasone and aldosterone increased the numbers of NSCs and induced differentiation of these cells into neurons. Dexamethasone also stimulated their differentiation into astrocytes. We did not examine the effects of these steroids on the differentiation of NSCs into oligodendrocytes. Previous reports indicated that MR activation was protective to granular cell neurons of the hippocampus, while persistent GR stimulation was toxic to these cells [31, 36]. Further, glucocorticoids induce apoptosis of neurons and neural precursor cells in the dentate gyrus of the rat hippocampus as well as the corresponding primary cell cultures, while they inhibit growth of astrocytes in this brain area [37, 38]. These effects of MR and GR on hippocampal neurons may ultimately affect memory consolidation and alterations in mood, cognition and activity of the HPA axis [31]. Thus, our results may provide important information on glucocorticoid-mediated regulation of neurons and astrocytes, underlying in part the known effect of glucocorticoids on neuronal cell fate, and memory consolidation and retrieval. However, it should be kept in mind that our NSCs were obtained from the embryonic brain cortex and were maintained *in vitro*, in contrast to hippocampal neurons *in vivo*, which have numerous cellular connections with surrounding and distant cells and are exposed to extracellular bioactive molecules secreted in a paracrine and/or autocrine fashion.

The mRNA and protein expression of several HDACs, including HDAC11, was highly regulated upon NSC differentiation. The HDAC inhibitor TSA decreased numbers of NSCs, but did not influence their differentiation. There are 4 subgroups of HDACs and they influence diverse functions of CNS by deacetylating many molecules localized at different subcellular compartments [39]. Our results are consistent with previous reports that HDAC-mediated transcriptional repression was essential for the proliferation and self-renewal of NSCs through cooperation with the Tailless (TLX) orphan nuclear receptor [40, 41]. Valproic acid, a compound frequently used as a mood stabilizer and as an anticonvulsant, is a HDAC inhibitor [42]. Further, several other HDAC inhibitors alter the fate of neural cells, and are now intensive research targets as potential therapeutic agents for several neurodegenerative diseases, cognitive disorders, multiple sclerosis and CNS trauma/injuries

[39, 43 – 45]. Thus, it is quite interesting to elucidate the exact roles of HDAC subtypes on the functions/differentiation of NSCs.

In summary, many NRs and their coregulators are differentially expressed in NSCs and show characteristic changes in their expression during the process of their differentiation. These molecules regulate diverse cellular activities after binding to their ligands, changing the expression of their target genes. Compounds, such as glucocorticoids, gonadal steroids and endocrine disruptors, derived from mothers either by endogenous production or acquisition from the environment, or secreted inside the fetus, could influence epigenetically the development of the CNS and its function after birth by affecting NSC self-renewal and differentiation into neuron and glial cells. These compounds might have potent therapeutic importance for neurodegenerative diseases and CNS injury.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

- Burris, TP. The nuclear receptor superfamily. In: Burris, TP.; McCabe, ERB., editors. Nuclear receptors and genetic disease. London: Academic Press; 2001. p. 1-58.
- Horn S, Heuer H. Thyroid hormone action during brain development: More questions than answers. *Mol Cell Endocrinol.* 2010; 315:19–26. [PubMed: 19765631]
- Wolkowitz OM, Burke H, Epel ES, Reus VI. Glucocorticoids. Mood, memory, and mechanisms. *Ann NY Acad Sci.* 2009; 1179:19–40. [PubMed: 19906230]
- Hughes ZA, Liu F, Marquis K, Muniz L, Pangalos MN, Ring RH, Whiteside GT, Brandon NJ. Estrogen receptor neurobiology and its potential for translation into broad spectrum therapeutics for CNS disorders. *Curr Mol Pharmacol.* 2009; 2:215–236. [PubMed: 20021460]
- Kino, T.; Chrousos, GP. Glucocorticoid effect on gene expression. In: Steckler, T.; Kalin, NH.; Reul, JMHM., editors. Handbook on Stress and the Brain. Amsterdam: Elsevier BV; 2005. p. 295-312.
- McKenna NJ, Lanz RB, O'Malley BW. Nuclear receptor coregulators: cellular and molecular biology. *Endocr Rev.* 1999; 20:321–344. [PubMed: 10368774]
- Kino T, Hurt DE, Ichijo T, Nader N, Chrousos GP. Noncoding RNA gas5 is a growth arrest- and starvation-associated repressor of the glucocorticoid receptor. *Sci Signal.* 2010; 3:ra8. [PubMed: 20124551]
- Androutsellis-Theotokis A, Murase S, Boyd JD, Park DM, Hoepfner DJ, Ravin R, McKay RD. Generating neurons from stem cells. *Methods Mol Biol.* 2008; 438:31–38. [PubMed: 18369747]
- Androutsellis-Theotokis A, Rueger MA, Park DM, Mkhikian H, Korb E, Poser SW, Walbridge S, Munasinghe J, Koretsky AP, Lonser RR, McKay RD. Targeting neural precursors in the adult brain rescues injured dopamine neurons. *Proc Natl Acad Sci USA.* 2009; 106:13570–13575. [PubMed: 19628689]
- Androutsellis-Theotokis A, Rubin de Celis MF, Ehrhart-Bornstein M, Bornstein SR. Common features between chromaffin and neural progenitor cells. *Mol Psychiatry.* 2012; 17:351. [PubMed: 22446576]

11. Bornstein SR, Ehrhart-Bornstein M, Androutsellis-Theotokis A, Eisenhofer G, Vukicevic V, Licinio J, Wong ML, Calissano P, Nistico G, Preziosi P, Levi-Montalcini R. Chromaffin cells: the peripheral brain. *Mol Psychiatry*. 2012; 17:354–358. [PubMed: 22249377]
12. Androutsellis-Theotokis A, Leker RR, Soldner F, Hoepfner DJ, Ravin R, Poser SW, Rueger MA, Bae SK, Kittappa R, McKay RD. Notch signalling regulates stem cell numbers in vitro and in vivo. *Nature*. 2006; 442:823–826. [PubMed: 16799564]
13. Androutsellis-Theotokis A, Rueger MA, Mkhikian H, Korb E, McKay RD. Signaling pathways controlling neural stem cells slow progressive brain disease. *Cold Spring Harb Symp Quant Biol*. 2008; 73:403–410. [PubMed: 19022746]
14. Kittappa R, Bornstein SR, Androutsellis-Theotokis A. The role of eNSCs in neurodegenerative disease. *Mol Neurobiol*. 2012 in press.
15. Ng SS, Chang TH, Tailor P, Ozato K, Kino T. Virus-induced differential expression of nuclear receptors and coregulators in dendritic cells: Implication to interferon production. *FEBS Lett*. 2011; 585:1331–1337. [PubMed: 21492741]
16. Kino T, Ichijo T, Amin ND, Kesavapany S, Wang Y, Kim N, Rao S, Player A, Zheng YL, Garabedian MJ, Kawasaki E, Pant HC, Chrousos GP. Cyclindependent kinase 5 differentially regulates the transcriptional activity of the glucocorticoid receptor through phosphorylation: Clinical Implications for the nervous system response to glucocorticoids and stress. *Mol Endocrinol*. 2007; 21:1552–1568. [PubMed: 17440046]
17. van Neerven S, Kampmann E, Mey J. RAR/RXR and PPAR/RXR signaling in neurological and psychiatric diseases. *Prog Neurobiol*. 2008; 85:433–451. [PubMed: 18554773]
18. Pereira FA, Qiu Y, Tsai MJ, Tsai SY. Chicken ovalbumin upstream promoter transcription factor (COUP-TF) expression during mouse embryogenesis. *J Steroid Biochem Mol Biol*. 1995; 53:503–508. [PubMed: 7626501]
19. Levesque D, Rouillard C. Nur77 and retinoid X receptors: Crucial factors in dopamine-related neuroadaptation. *Trends Neurosci*. 2007; 30:22–30. [PubMed: 17134767]
20. Hall MG, Quignodon L, Desvergne B. Peroxisome proliferator-activated receptor γ in the brain: Facts and hypothesis. *PPAR Res*. 2008; 2008:780452. [PubMed: 19009042]
21. Lee YF, Lee HJ, Chang C. Recent advances in the TR2 and TR4 orphan receptors of the nuclear receptor superfamily. *J Steroid Biochem Mol Biol*. 2002; 81:291–308. [PubMed: 12361719]
22. Schnegg CI, Robbins ME. Neuroprotective mechanisms of PPAR γ : Modulation of oxidative stress and inflammatory processes. *PPAR Res*. 2011; 2011:373560. [PubMed: 22135673]
23. Real MA, Heredia R, Davila JC, Guirado S. Efferent retinal projections visualized by immunohistochemical detection of the estrogen-related receptor α in the postnatal and adult mouse brain. *Neurosci Lett*. 2008; 438:48–53. [PubMed: 18472334]
24. Grgurevic N, Tobet S, Majdic G. Widespread expression of liver receptor homolog 1 in mouse brain. *Neuro Endocrinol Lett*. 2005; 26:541–547. [PubMed: 16264403]
25. Seckl JR, Walker BR. Minireview: 11 β -hydroxysteroid dehydrogenase type 1—a tissue-specific amplifier of glucocorticoid action. *Endocrinology*. 2001; 142:1371–1376. [PubMed: 11250914]
26. Almad A, McTigue DM. Chronic expression of PPAR- γ by oligodendrocyte lineage cells in the injured rat spinal cord. *J Comp Neurol*. 2010; 518:785–799. [PubMed: 20058304]
27. D'Angelo B, Benedetti E, Di Loreto S, Cristiano L, Laurenti G, Ceru MP, Cimini A. Signal transduction pathways involved in PPAR γ -induced neuronal differentiation. *J Cell Physiol*. 2011; 226:2170–2180. [PubMed: 21520069]
28. Schnegg CI, Kooshki M, Hsu FC, Sui G, Robbins ME. PPAR γ prevents radiation-induced proinflammatory responses in microglia via transrepression of NF- κ B and inhibition of the PKC δ / MEK1/2/ERK1/2/AP-1 pathway. *Free Radic Biol Med*. 2012; 52:1734–1743. [PubMed: 22387176]
29. Siegel DA, Davies P, Dobrenis K, Huang M. Tomoregulin-2 is found extensively in plaques in Alzheimer's disease brain. *J Neurochem*. 2006; 98:34–44. [PubMed: 16805794]
30. Zhou XE, Suino-Powell KM, Xu Y, Chan CW, Tanabe O, Kruse SW, Reynolds R, Engel JD, Xu HE. The orphan nuclear receptor TR4 is a vitamin A-activated nuclear receptor. *J Biol Chem*. 2011; 286:2877–2885. [PubMed: 21068381]

31. Sousa N, Almeida OF. Corticosteroids: Sculptors of the hippocampal formation. *Rev Neurosci*. 2002; 13:59–84. [PubMed: 12013026]
32. Kino T, Chrousos GP. Glucocorticoid and mineralocorticoid resistance/hypersensitivity syndromes. *J Endocrinol*. 2001; 169:437–445. [PubMed: 11375113]
33. Chrousos, GP. Glucocorticoid therapy. In: Felig, P.; Frohman, LA., editors. *Endocrinology & Metablism*. 4. New York: McGraw-Hill; 2001. p. 609-632.
34. Aimone JB, Wiles J, Gage FH. Potential role for adult neurogenesis in the encoding of time in new memories. *Nat Neurosci*. 2006; 9:723–727. [PubMed: 16732202]
35. Kukolja J, Thiel CM, Wolf OT, Fink GR. Increased cortisol levels in cognitively challenging situations are beneficial in young but not older subjects. *Psychopharmacology (Berl)*. 2008; 201:293–304. [PubMed: 18704371]
36. Gass P, Kretz O, Wolfer DP, Berger S, Tronche F, Reichardt HM, Kellendonk C, Lipp HP, Schmid W, Schutz G. Genetic disruption of mineralocorticoid receptor leads to impaired neurogenesis and granule cell degeneration in the hippocampus of adult mice. *EMBO Rep*. 2000; 1:447–451. [PubMed: 11258486]
37. Yu S, Yang S, Holsboer F, Sousa N, Almeida OF. Glucocorticoid regulation of astrocytic fate and function. *PLoS One*. 2011; 6:e22419. [PubMed: 21811605]
38. Yu S, Patchev AV, Wu Y, Lu J, Holsboer F, Zhang JZ, Sousa N, Almeida OF. Depletion of the neural precursor cell pool by glucocorticoids. *Ann Neurol*. 2010; 67:21–30. [PubMed: 20186952]
39. Kazantsev AG, Thompson LM. Therapeutic application of histone deacetylase inhibitors for central nervous system disorders. *Nat Rev Drug Discov*. 2008; 7:854–868. [PubMed: 18827828]
40. Sun G, Yu RT, Evans RM, Shi Y. Orphan nuclear receptor TLX recruits histone deacetylases to repress transcription and regulate neural stem cell proliferation. *Proc Natl Acad Sci USA*. 2007; 104:15282–15287. [PubMed: 17873065]
41. Sun G, Fu C, Shen C, Shi Y. Histone deacetylases in neural stem cells and induced pluripotent stem cells. *J Biomed Biotechnol*. 2011; 2011:835968. [PubMed: 21845024]
42. Chateavieux S, Morceau F, Dicato M, Diederich M. Molecular and therapeutic potential and toxicity of valproic acid. *J Biomed Biotechnol*. 2010; 2010:479364. [PubMed: 20798865]
43. Faraco G, Cavone L, Chiarugi A. The therapeutic potential of HDAC inhibitors in the treatment of multiple sclerosis. *Mol Med*. 2011; 17:442–447. [PubMed: 21373721]
44. Fischer A, Sananbenesi F, Mungenast A, Tsai LH. Targeting the correct HDAC(s) to treat cognitive disorders. *Trends Pharmacol Sci*. 2010; 31:605–617. [PubMed: 20980063]
45. Shein NA, Shohami E. Histone deacetylase inhibitors as therapeutic agents for acute central nervous system injuries. *Mol Med*. 2011; 17:448–456. [PubMed: 21274503]

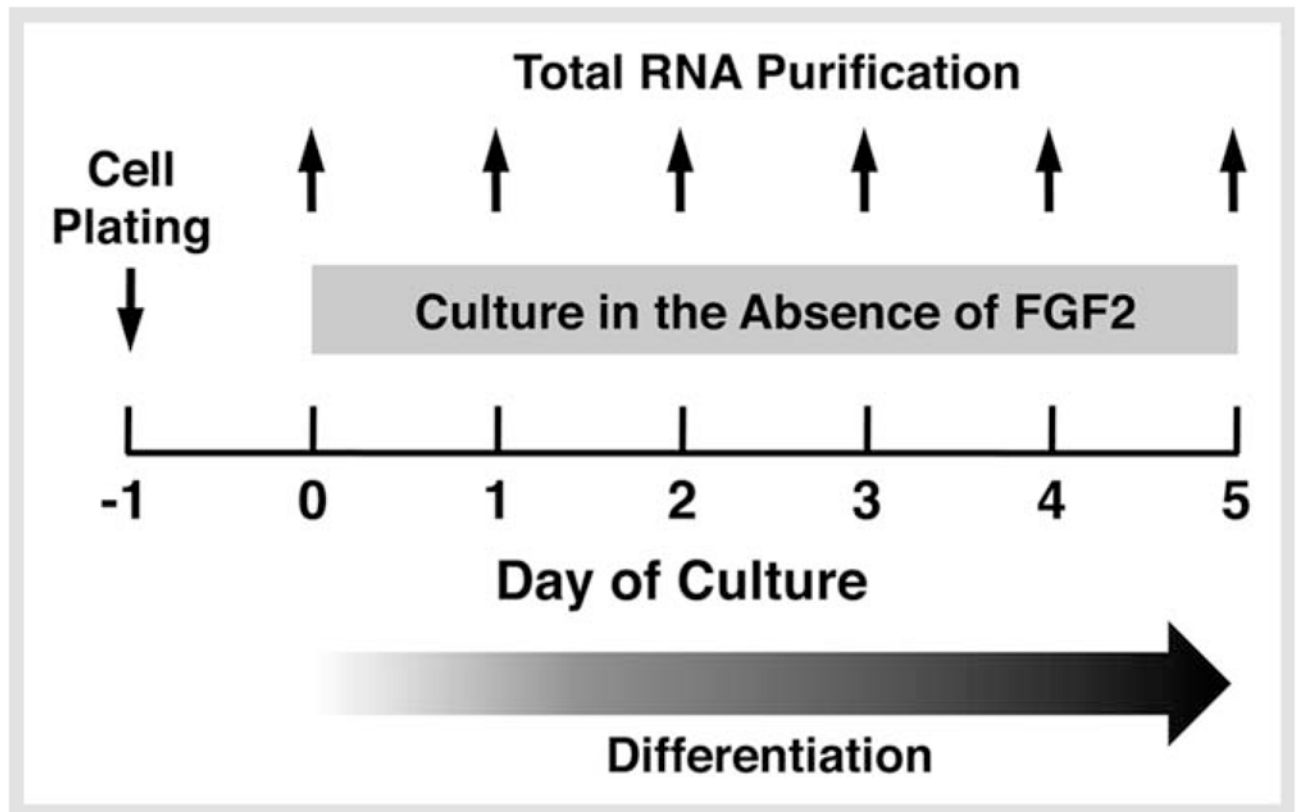


Fig. 1. Experimental procedures for evaluating NR/coregulator mRNA expression profiles upon differentiation of NSCs. NSCs were plated one day before FGF2 withdrawal. They were then cultured in the absence of FGF2 for 5 days. Total RNA was extracted from the cells daily for examining mRNA expression of NRs and their coregulators using the custom PCR arrays.

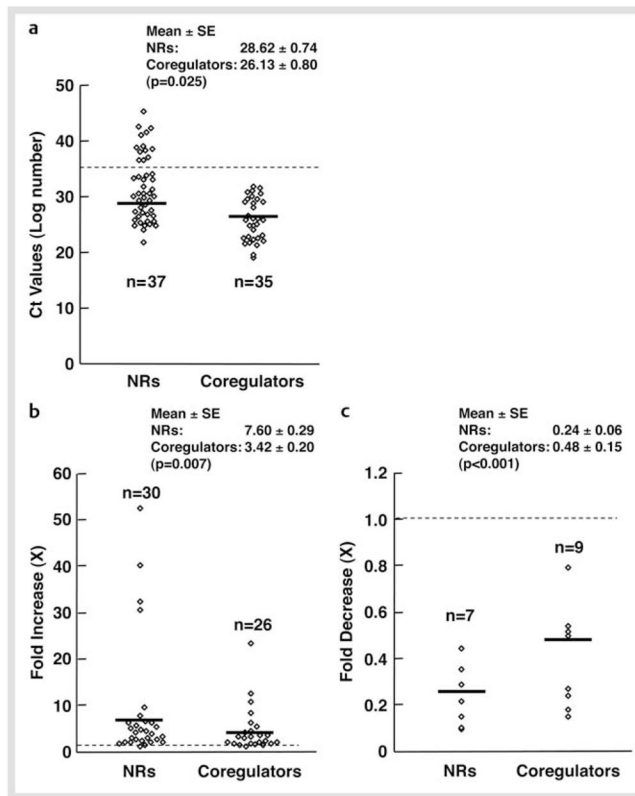


Fig. 2. mRNA expression of 49 NRs and coregulators in NSCs at the baseline and fold changes after FGF2 withdrawal. **a** The mRNA of 37 NRs receptors and 35 coregulators is expressed in NSCs. Thirty seven NRs and all coregulators examined were expressed in mouse NSCs at baseline, under the criterion that the Ct value ≤ 35 is the lowest limit for expression (shown as a dashed line). Their Ct values are shown as open circles, while mean values are indicated with bold horizontal lines. mRNA expression profiles of NRs and coregulators at the baseline condition were used to create this Figure. **b** and **c** Fold increase **b** and decrease **c** of mRNA expression of NRs and coregulators in NSCs before and after FGF2 withdrawal. Fold changes in the Ct values of expressed NRs and coregulators are shown as open circles, while their mean values are indicated with bold horizontal lines. Dashed line indicates the fold change of “1”. Maximum fold changes found in the mRNA expression profiles of NRs and coregulators upon FGF2 withdrawal were used to create this Figure.

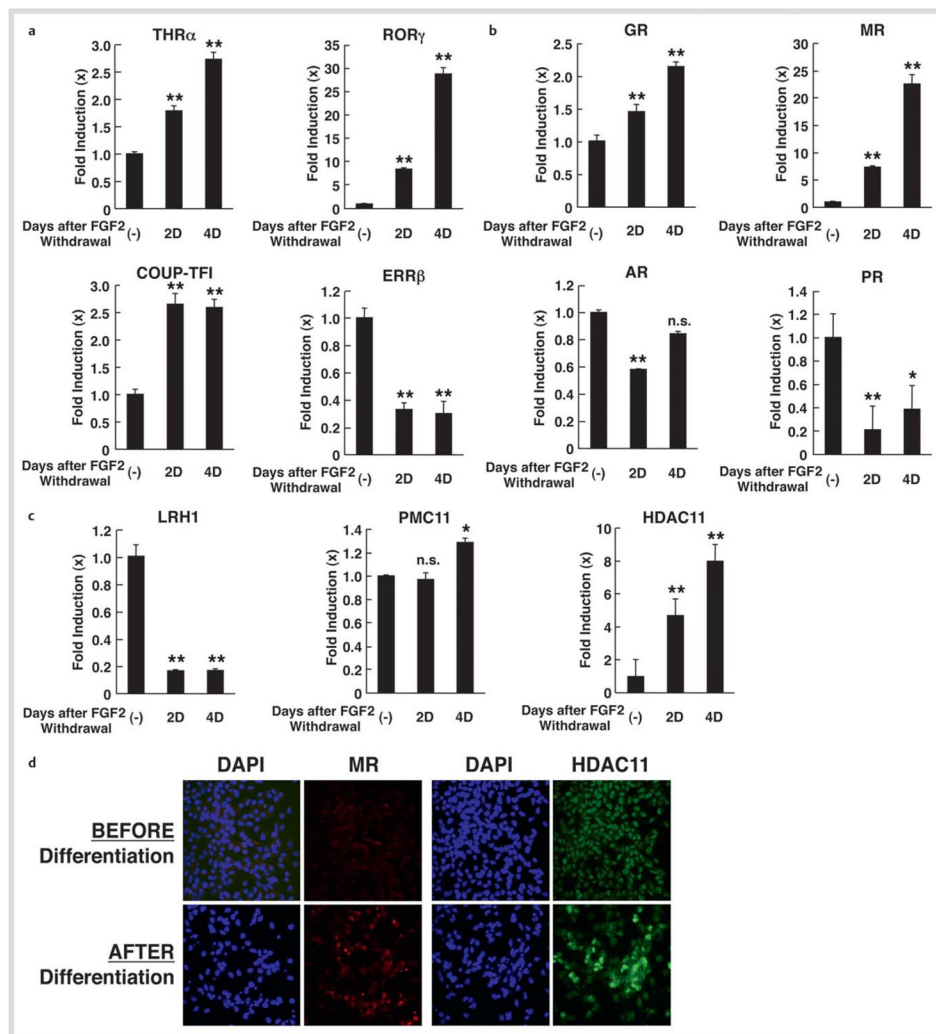
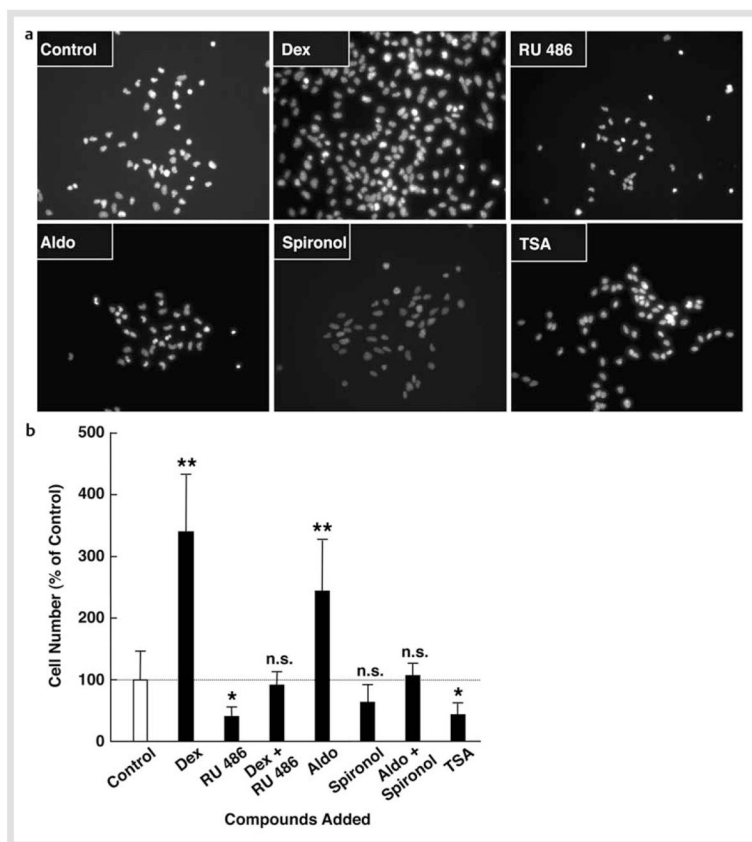


Fig. 3. mRNA and protein expression of selected NRs and HDAC11 in NSCs before and after FGF2 withdrawal. **a**, **b**, and **c** mRNA expression of selected NRs and HDAC11 in NSCs upon FGF2 withdrawal. mRNA expression of THR, ROR, COUP-TFI and ERR **a**, GR, MR, AR and PR **b**, and LRH1, PMC11, and HDAC11 **c** were examined with the SYBR Green-based real-time PCR using their specific primers listed in Table 1. Total RNA samples employed in Fig. 1 were used. Bars represent the mean \pm S.E. values of fold mRNA expression upon FGF2 withdrawal compared with baseline (in the absence of FGF2 withdrawal). **D**: days after FGF2 withdrawal. * $p < 0.05$, ** $p < 0.01$, n. s.: not significant, compared to baseline. **d** Protein expression of MR and HDAC11 in NSCs upon FGF2 withdrawal. NSCs before and after incubation for 5 days in the absence of FGF2 were immunostained with anti-MR or -HDAC11 antibody, and subsequently, by appropriate fluorescence-conjugated secondary antibodies. Cells were also stained with DAPI.

**Fig. 4.**

Dexamethasone and aldosterone increase NSC numbers, while TSA suppresses them. NSCs were cultured in the presence of 10^{-6} M of dexamethasone (Dex) and/or RU 486, 10^{-8} M of aldosterone (Aldo) and/or spironolactone (Spironol), or 10^{-6} M of trichostatin-A (TSA) in the presence of FGF2 for 2 days. Cells were then fixed and stained with DAPI and their images were taken or the numbers of NSCs were counted under fluorescence microscopy. Images of DAPI staining are shown in **a**, while percent increases of cell numbers compared to baseline (the cells before FGF2 withdrawal) are shown in **b**. Bars indicate mean \pm S.E. values of % cell numbers compared to control. * $p < 0.05$, ** $p < 0.01$, n. s.: not significant, compared to control.

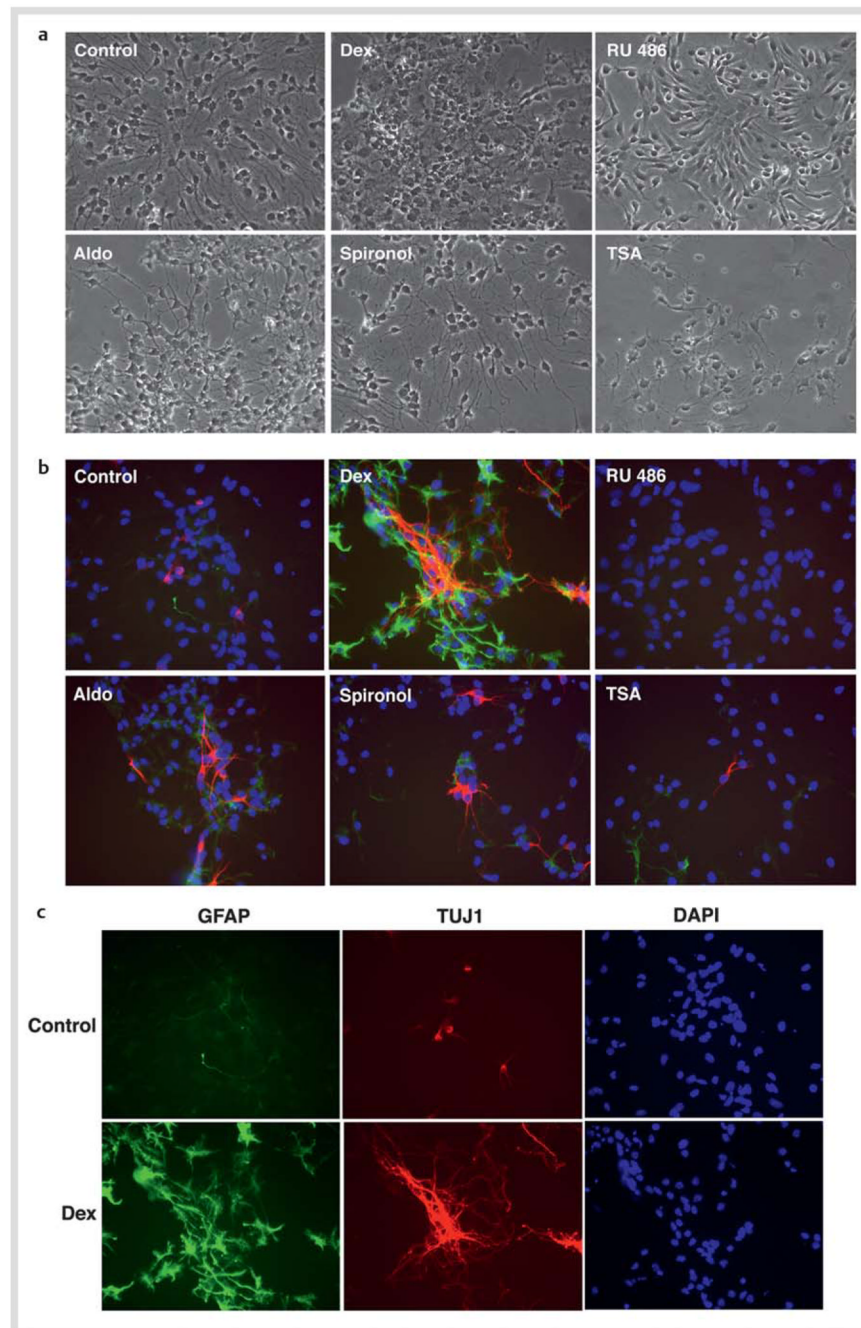


Fig. 5. Dexamethasone and aldosterone influence differentiation of NSCs. NSCs were cultured in the presence of 10^{-6} M of dexamethasone (Dex) or RU 486, 10^{-8} M of aldosterone (Aldo) or spironolactone (Spironol), or 10^{-6} M of trichostatin-A (TSA) for 2 days in the absence of FGF2, and were fixed for staining of GFAP (astrocytes: green) and TUJ1 (neurons: red). Nomarski images are shown in **a**, while images of immunostaining for GFAP and TUJ1 together with DAPI staining are shown in **b**. **c** demonstrates results of individual images of GFAP, TUJ1, and DAPI staining for the cells cultured in the presence or absence of dexamethasone.

Table 1

Primer pairs used in the SYBR Green-based real-time PCR.

Gene name	Primer sequence	
AR	Forward	5 -CAGAAGATGACTGTATCAC-3
	Reverse	5 -CTAGATAACAAGGCAGCAAAGG-3
COUP-TFI	Forward	5 -GTCGACTCTGCCGAGTACAG-3
	Reverse	5 -CAGGGCACACTGTGATTCTC-3
ERR	Forward	5 -GTGGTGGCTTTGGCATTGC-3
	Reverse	5 -CATGTA CTGCATTGATG-3
GR	Forward	5 -GTTCTAAGGAAGGTCTGAAGAG-3
	Reverse	5 -CAATTCTGACTGGAGTTCC-3
HDAC11	Forward	5 -GAGCCACCATCATTGATCTC-3
	Reverse	5 -GATGGCCTCTTAGCAAAGC-3
LRH1	Forward	5 -CGAAGATCATGGCTTACC-3
	Reverse	5 -GTTCCCTGAAGAAGATACTACTC-3
MR	Forward	5 -GTGTGCTGGAAGAAATGAC-3
	Reverse	5 -CAGCTTCTTTGACTTTCG-3
PMC11	Forward	5 -GCTGTAATGCAAATGGTAAAGC-3
	Reverse	5 -CAGTGTGTGTGCACTTACC-3
PR	Forward	5 -CCAGATAACCCTGATTCAG-3
	Reverse	5 -CTGCTCATTTAGGATTAGATCAG-3
ROR	Forward	5 -GAGGAGAGTGAACATCTG-3
	Reverse	5 -CTGCAGCTTTCCACATGTTG-3
THR	Forward	5 -GTGCTGCTAATGTCAACAG-3
	Reverse	5 -GTTGTGTTTGCGGTGGTTGAC-3
RPLP0	Forward	5 -GAGGACCTCACTGAGATTCG-3
	Reverse	5 -CTGGAAGAAGGAGTCTTCTC-3

AR: androgen receptor; CAR: constitutive androstane receptor; COUP-TFI: chicken ovalbumin upstream promoter-transcription factor I; ERR : estrogen-related receptor ; GR: glucocorticoid receptor; HDAC11: histone deacetylase 11; LRH1: liver receptor homologue 1; MR: mineralocorticoid receptor; PMC11: progesterone membrane component 11; PR: progesterone receptor; ROR : RAR-related orphan receptor ; THR : thyroid hormone receptor ; RPLP0: acidic ribosomal phosphoprotein P0.

Table 2

Ct values of NRs and coregulators in NSCs.

Subfamilies	Genes	Name	Symbol	Mean	SE
Nuclear Receptors					
1	1A	NR1A1	Thyroid hormone receptor	THR	25.41 0.379
2		NR1A2	Thyroid hormone receptor	THR	30.97 0.645
3	1B	NR1B1	Retinoic acid receptor	RAR	45.73 1.253
4		NR1B2	Retinoic acid receptor	RAR	39.64 0.281
5		NR1B3	Retinoic acid receptor	RAR	28.58 0.142
6	1C	NR1C1	Peroxisome proliferator-activated receptor	PPAR	30.33 0.171
7		NR1C2	Peroxisome proliferator-activated receptor	PPAR	25.88 0.040
8		NR1C3	Peroxisome proliferator-activated receptor	PPAR	38.88 0.179
9	1F	NR1F1	RAR-related orphan receptor	ROR	26.28 0.126
10		NR1F2	RAR-related orphan receptor	ROR	27.3 1.673
11		NR1F3	RAR-related orphan receptor	ROR	34.3 0.195
12	1H	NR1H2	Liver X receptor	LXR	29.15 0.085
13		NR1H3	Liver X receptor	LXR	29.75 0.065
14		NR1H4	Farnesoid X receptor	FXR	39.28 0.538
15		NR1H5	Farnesoid X receptor	FXR	38.66 2.041
16	1I	NR1I1	Vitamin D receptor	VDR	37.05 0.404
17		NR1I2	Pregnane X receptor	PXR	31.16 0.220
18		NR1I3	Constitutive androstane receptor	CAR	25.46 0.081
19	2A	NR2A1	Hepatic nuclear receptor 4	HNF4	33.61 0.077
20		NR2A2	Hepatic nuclear receptor 4	HNF4	42.93 0.306
21	2B	NR2B1	Retinoid X receptor	RXR	27.63 1.139
22		NR2B2	Retinoid X receptor	RXR	24.60 0.208
23		NR2B3	Retinoid X receptor	RXR	33.66 0.996
24	2C	NR2C1	Testicular receptor 2	TR2	26.01 0.396
25		NR2C2	Testicular receptor 4	TR4	22.41 0.008
26	2E	NR2E2	Tailless	TLX	31.06 0.224

Subfamilies	Genes	Name	Symbol	Mean	SE
27	NR3E3	Photospecific nuclear receptor	PNR	34.42	0.285
28	2F	Chicken ovalbumin upstream promoter-TFI	COUP-TFI	26.85	1.530
29	NR2F2	Chicken ovalbumin upstream promoter-TFII	COUP-TFII	26.37	0.053
30	NR2F6	v-ErbA related 2	EAR2	25.25	0.012
31	3A	NR3A1	ER	41.43	0.706
32	NR3A2	Estrogen receptor	ER	33.91	0.489
33	NR3B1	Estrogen-related receptor	ERR	42.7	2.114
34	3B	NR3B2	ERR	32.31	0.126
35	NR3B3	Estrogen-related receptor	ERR	38.99	0.396
36	3C	NR3C1	GR	27.95	0.306
37	NR3C2	Mineralocorticoid receptor	MR	37.07	0.130
38	NR3C3	Progesterone receptor	PR	30.58	0.097
39	NR3C4	Androgen receptor	AR	27.00	0.106
40	4A	NR4A1	NUR77	25.29	0.097
41	NR4A2	Nuclear receptor-related 1	NURR1	27.78	0.073
42	NR4A3	Neuron-derived orphan receptor 1	NOR1	42.05	6.487
43	5A	NR5A1	SF1	34.05	0.032
44	NR5A2	Liver receptor homologue 1	LRH1	29.91	0.093
45	6A	NR6A1	GCNFI	30.53	0.077
46	0B	NR0B1	DAX1	31.83	0.220
47	NR0B2	Small heterodimer partner	SHP	37.48	0.167
Membrane nuclear receptors					
1		Progesterone membrane component 11	PMC11	26.44	0.024
2		P Protein-coupled estrogen receptor 30	GOCR30	39.69	1.686
Coregulators					
1		CREB-binding protein	CBP	22.77	0.110
2		p300	p300	26.42	0.016
3		Nuclear receptor coactivator 1	NCoA1	25.21	0.016
4		Nuclear receptor coactivator 2	NCoA2	22.73	0.008

Subfamilies	Genes	Name	Symbol	Mean	SE
5		Nuclear receptor coactivator 3	NCoA3	24.61	0.073
6		p300/CBP-associated factor	p/CAF	28.45	0.318
7		Nuclear receptor-interacting protein 1	NRIP1	30.95	0.016
8		PPAR coactivator 1	PGC1	26.56	0.183
9		Nuclear hormone receptor coregulator 6	NRC6	32.37	0.044
10		Structural maintenance of chromosome protein 1 (SMC)	SMC1	29.4	0.383
11		Structural maintenance of chromosomes protein 4	SMC4	29.54	0.457
12		Thyroid receptor-associated protein/complex 220 kDa component	TRAP220	23.07	0.089
13		Thyroid hormone receptor-associated protein complex 170 kDa component	TRAP170	23.20	0.526
14		Mediator of RNA polymerase II transcription subunit 6	MED6	27.07	1.355
15		Histone deacetylase 1	HDAC1	22.35	0.024
16		Histone deacetylase 2	HDAC2	30.02	0.685
17		Histone deacetylase 3	HDAC3	22.01	0.061
18		Histone deacetylase 4	HDAC4	30.52	0.028
19		Histone deacetylase 6	HDAC6	25.27	0.044
20		Histone deacetylase 7A	HDAC7	25.18	0.032
21		Histone deacetylase 10	HDAC10	31.39	0.269
22		Histone deacetylase 11	HDAC11	31.54	0.216
23		Nuclear receptor corepressor 1	NCoR1	25.56	0.069
24		Nuclear receptor corepressor 2	NCoR2	29.98	0.069
25		SIN3 homolog	Sin3A	24.64	0.469
26		Set/Template activating factor 1	Set/TAF-I	22.93	0.004
27		Coactivator-associated arginine methyltransferase-1	CARM1	21.82	0.032
28		HRMT1-like 2	HRMT-like 2	22.43	0.171
29		Suppressor of variegation 3-9 homolog 1	SUV39HI	32.01	1.481
30		C-terminal tail-binding protein 1	CTBP1	19.63	0.036
31		Growth arrest-specific 5	Gas5	26.27	0.881
32		Steroid receptor RNA coactivator	SRA	26.35	0.351
33		SNF2 histone linker PHD RING helicase	SHPRH	23.49	0.077

Subfamilies	Genes	Name	Symbol	Mean	SE
34		SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4	SMARCA4	20.13	0.032
35		Brx (PRKA13) Rho-type GEF	AKAP13	29.47	0.012
Controls					
1		GUSB		23.53	0.0938
2		HPT1		22.22	0.0163
3		HSP1b		16.92	0.232
4		GAPDH		16.97	0.191
5		-Actin		15.25	0.1633

Unexpressed molecules ($Ct > 35$) are shown in grey

Table 3

Percentages of indicated cell lineages in NSC cultured in the absence of FGF2 for 2 days.

Treatment	Undifferentiated cells	Cell types (means±S.D.) (% of total)	Neurons	Astrocytes
No steroids	77.71±6.67	7.86±2.88		14.39±5.13
Dexamethasone	20.12±3.09**	19.31±3.82*		60.58±7.46**
Aldosterone	67.15±5.28	14.47±0.62*		18.37±5.45

* p < 0.05,

** p < 0.01, compared to the baseline (no steroids)