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Distribution of corticosteroid receptors in mature oligodendrocytes and oligodendrocyte progenitors of the adult mouse brain

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Running title: Corticosteroid receptors in oligodendrocytes

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4 **Distribution of corticosteroid receptors in mature oligodendrocytes and**
5 **oligodendrocyte progenitors of the adult mouse brain**
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Abstract

The expression of glucocorticoid receptors (GRs) was investigated immunohistochemically in two different lineages of oligodendrocytes, using carbonic anhydrase (CA) II and neuron glial antigen (NG) 2 as markers of mature oligodendrocytes and oligodendrocyte progenitors, respectively. We focused on the gray matter regions, including CA1, CA3 and the dentate gyrus of the hippocampus, the primary somatosensory cortex barrel field and the basolateral amygdala, and the white matter regions, including the corpus callosum, external capsule and fimbria of the hippocampus. More than 80% of CAII-immunoreactive (IR) cells and more than 85% of NG2-IR cells expressed GRs in various regions of the brain. In contrast, neither CAII-IR cells nor NG2-IR cells expressed mineralocorticoid receptors (MRs) in the same regions. The intensity of GR expression was drastically reduced in CA II-IR cells and NG2-IR cells in the same regions in adrenalectomized mice. Finally, steroid receptor co-activator (SRC)-1 and p300, both of which are cofactors for GR, were expressed in the gray and white matter regions in NG2-IR cells, but not in CAII-IR cells. These results suggest that the expression of GRs in oligodendrocytes and their

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5 progenitor cells mediates several functions *in vivo*, including differentiation and
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8 myelination, as a major target of glucocorticoids and their cofactors.
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15 **Key words**

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18 Glucocorticoid receptor, oligodendrocyte, oligodendrocyte progenitor, corticosteroid
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21 (glucocorticoid), mineralocorticoid receptor, cofactor
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Introduction

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Glucocorticoids contribute to a wide range of actions in the nervous system, including mediating stress responses, energy metabolism, cell growth and differentiation, and immune and inflammatory responses (Sapolsky et al. 2000; de Kloet et al. 2005). These functions are exerted through two receptor targets, the glucocorticoid receptors (GRs) and mineralocorticoid receptors (MRs), which bind the same hormone (primarily cortisol in humans and corticosterone in rodents) and work as ligand-dependent transcription factors to exert classical glucocorticoid actions (Han et al. 2005). In contrast, rapid, non-genomic actions of glucocorticoids have also been reported (Cintra et al. 1994). The classical hormone actions are regulated by a subset of nuclear proteins called cofactors (coactivators and corepressors), such as steroid receptor coactivator 1 (SRC-1) and p300 (Leo et al. 2000; Wu RC et al. 2005). Corticosteroids are widely used in the treatment of various kinds of diseases, including neurodegenerative diseases that affect white matter, such as multiple sclerosis (MS) (Myhr and Mellgren. 2009), whereas corticosteroids are ineffective in gray matter injuries, such as head injury and stroke (Sun et al. 2010). Furthermore, recent studies have demonstrated that corticosteroids are involved in various aspects of the regulation

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5 of oligodendrocytes, including the proliferation, differentiation, and protection of
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8 oligodendrocytes, independent of the treatment of multiple sclerosis. For example, it
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11 was recently demonstrated that plasma corticosterone activates SGK1 and induces
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14 morphological changes in oligodendrocytes (Miyata et al. 2011). Glucocorticoids also
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17 protect oligodendrocytes against excitotoxin (Sun et al. 2010). Thus, it would be
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21 interesting to elucidate whether or not the distribution patterns of GR in
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24 oligodendrocytes in both white matter and gray matter are different.
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28 Although many studies have reported GR expression in cultured oligodendrocytes
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31 (Bohn et al. 1991), very few investigations have reported GR expression in
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34 oligodendrocytes *in vivo*. The primary purpose of the present study was to investigate
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37 the expression of GRs and GR cofactors in oligodendrocytes in the brains of adult mice.
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40 To this end, we performed three major experiments, as follows. First, the expression of
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43 GRs in oligodendrocytes was investigated immunohistochemically in various regions of
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46 the brain in adult mice. Since GRs are involved in cell growth and differentiation in the
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49 central nervous system (CNS), the expression pattern of GRs in different lineages of
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52 oligodendrocytes would be helpful in understanding this role. We selected two markers
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55 for oligodendrocytes, carbonic anhydrase (CA) II and neuron glial antigen (NG) 2. CAII
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5 is one of seven CA isozymes that are expressed in CNS and is considered as a marker of
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8 adult oligodendrocytes (Ghandour et al. 1980). In contrast, NG2 is an integral
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11 membrane chondroitin sulfate proteoglycan, which is expressed on oligodendrocyte
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14 progenitors (Nishiyama et al. 1996). We analyzed the gray matter regions, including
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17 CA1, CA3 and the dentate gyrus in the hippocampus, primary somatosensory cortex
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20 barrel field and basolateral amygdala, and on the white matter regions, including the
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23 corpus callosum, external capsule and fimbria of the hippocampus. The expression of
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26 another corticosteroid receptor, MR, was also investigated in oligodendrocytes in the
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29 same regions of the adult brain using the same oligodendrocyte markers. Next, the
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32 effect of reduction of corticosterone (CORT) levels on the expression of GRs in CAII-
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35 immunoreactive (IR) oligodendrocytes and NG2-IR oligodendrocyte progenitors was
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38 examined in adult mice that had undergone bilateral adrenalectomy to determine
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41 whether GRs expressed in oligodendrocytes are functional and responsive to its ligand,
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44 CORT. Finally, the expression of steroid receptor co-activator (SRC)-1 and p300 was
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47 investigated immunohistochemically in CAII-IR oligodendrocytes and NG2-IR
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50 oligodendrocyte progenitors, along with GR expression. The transcriptional activity of
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53 GRs is enhanced by the recruitment of one of the transcriptional co-activators of the
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5 p160 family (SRCs), with a docking platform for secondary co-activators, such as the
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8 cAMP-response element binding protein (CREB)-binding protein (CBP), or its close
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11 homologue p300. While glucocorticoids have been reported to promote myelination
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13 through SRC-1 or p300 in Schwann cells, it is not known whether SRC-1 and p300 are
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15 localized in oligodendrocytes (Désarnaud et al. 2000). Therefore, clarification of SRC-1
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18 and p300 expression in oligodendrocytes would help to determine whether GR function
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24 is regulated by these cofactors.
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28 The results demonstrate for the first time that GRs are expressed in different
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30 lineages of oligodendrocytes, mature CAII-IR oligodendrocytes and NG2-IR
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33 oligodendrocyte progenitors *in vivo*, and suggest that GRs mediate several important
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36 functions, including promotion of myelination, as a major target of glucocorticoids.
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43 44 **Material and Method**

45 46 47 **Animals**

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50 Adult male C57BL/6 mice (8-10 weeks old) were purchased from Japan SLC
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53 (Hamamatsu, Japan) and housed in groups (4 animals /case) in a room with a 12 hr
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56 light-dark cycle with the light turned on at 8:00 AM. Food pellets and drinking water
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5 were provided *ad libitum*. The protocols for this study were approved by the Animal
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8 Care Committee of Nara Medical University in accordance with the policies established
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11 by the NIH for the care and use of laboratory animals.
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18 **Preparation of the brain sections**

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21 Mice were deeply anesthetized in the morning (9:30-11:30 AM) by intraperitoneal
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23 injection of pentobarbital sodium salt solution (Somnopencyl 0.1ml/10g bodyweight;
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25 Kyoritsu Seiyaku Corporation, Tokyo, Japan) and then perfused intracardially with
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27 ice-cold phosphate-buffer saline (PBS) followed by 4% paraformaldehyde (PFA), pH7.4.
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33 After perfusion, the brains were removed and fixed in 4% PFA at 4°C overnight.
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37 Coronal sections were cut (50µm sections) with a linear slicer (Pro. 7, DOSAKA EM
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41 CO. (D.K.S.), Kyoto, Japan).
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47 **Adrenalectomy and corticosterone (CORT) assay**

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50 Six mice underwent bilateral adrenalectomy in the morning (9:30-11:30 AM) and
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53 another six mice underwent sham-operation for adrenalectomy. After the mice were
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56 anesthetized with isoflurane (Escaïn; Mylan Inc., Pittsburgh, USA), the skin of the right
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5 side of their flank was opened, the muscles dissected and the adrenal gland removed.
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8 The left adrenal gland was removed through the same skin incision. After
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11 adrenalectomy, the mice were provided drinking water supplemented with 0.9% saline.
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15 Three days after adrenalectomy, the animals were sacrificed under pentobarbital
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18 anesthesia. Blood samples were collected by intracardiac puncture and stored in
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21 heparinized tubes. Plasma was obtained by centrifugation (2,000g for 10min at 4°C) and
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24 stored at -80°C until the day for subsequent assay. The plasma concentrations of CORT,
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27 the rodent glucocorticoid, were measured using an EIA kit (YK240, Yanaihara Institute,
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30 Inc., Shizuoka, Japan) to confirm resection of the adrenal glands. After the blood
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34 samples were obtained, the animals were perfused with ice-cold PBS followed by PFA.
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37 The brains were removed and fixed with PFA. Coronal sections (50µm thick) were
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40 prepared as described above.
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47 **Immunohistochemistry**

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50 Free-floating coronal sections were washed with PBS, immersed in 25mM glycine
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53 in PBS for 20 min, and permeabilized with 0.3% Triton-X 100 in PBS (PBS-T) for 15
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56 min. After blocking in PBS-T supplemented with 5% normal goat serum (NGS) or 5%
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5 normal horse serum (NHS) at room temperature for 2 hours, rabbit polyclonal antibody
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8 against rat GR (1:1000; provided by Prof. M. Kawata [Morimoto et al. 1996]) and the
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11 mouse monoclonal antibody against rat MR (1:200; provided by Prof. C. E.
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14 Gomez-Sanchez [Gomez-Sanchez CE et al. 2006]) were used to detect GR- and
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17 MR-immunoreactivities, respectively. A sheep polyclonal antibody against human
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20 Carbonic Anhydrase II antibody (CAII, ab8953) (1:1000; Abcam, UK) was used to
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23 identify mature oligodendrocytes, and a polyclonal guinea pig antibody against rat NG2
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26 (1:200; provided by Dr. W. B. Stallcup, [Ozerdem et al. 2001; Stallcup et al. 2002;
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29 Kucharova et al. 2011]) was used to identify oligodendrocyte progenitors. The
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32 antibodies used to examine the cofactors in the oligodendrocytes included a polyclonal
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35 rabbit antibody against human SRC-1 (M-341) (1:500; Santa Cruz Biotechnology,
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38 USA), and a monoclonal mouse antibody against rat p300 (NM-11; 1:250; Calbiochem,
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41 Germany). Sections were incubated with primary antibodies (diluted in PBS-T) with 1%
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44 NHS at 4°C overnight. After washing, sections were incubated with either
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47 AlexaFluor-488 labeled donkey-anti-rabbit, AlexaFluor-488 labeled goat-anti-rabbit
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50 IgG, AlexaFluor-488 labeled donkey-anti-mouse IgG, AlexaFluor-488 labeled
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53 goat-anti-mouse IgG or AlexaFluor-546 labeled donkey-anti-sheep and AlexaFluor-546
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5 labeled goat-anti-guinea pig (all secondary antibodies 1:1000, Molecular Probes, USA)
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8 in PBS-T with 1% NHS for 2h at room temperature. After washing with PBS for 15min,
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10 sections were mounted and shielded with Vectashield® mounting media (Vector
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12 Laboratories, Burlingame, CA) containing 4', 6-diamino-2-phenylindole (DAPI), and
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15 stored in the dark until further analysis. Observation and capturing of images were
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18 carried out using a FluoView 1000 confocal microscope (Olympus, Tokyo, Japan).
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27 **Quantitative analysis**

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30 To quantify the percentages of GR- and MR-immunoreactivity in oligodendrocytes
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32 and to measure the fluorescent intensity of GR-immunoreactivity in oligodendrocytes
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34 from sham- and adrenalectomy-operated mice, brain sections were double-labeled with
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36 GR and CAII or GR and NG2, all of which were counterstained with DAPI. Sections at
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38 1.4-1.7 mm posterior to the Bregma according to the mouse brain atlas (Franklin and
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40 Paxinos, 2008) were used for all analyses. Analyzed regions were illustrated in Figure 1.
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Fluorescent images were captured using a confocal laser microscope (Olympus
Fluoview 1000) with the 40×objective lens. To count GR-IR cells in oligodendrocytes,
four images per region were obtained per mouse and the ratio of GR-IR cells in CAII-

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5 or NG2-IR cells was calculated. The total number of CAII-IR or NG2-IR positive cells
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8 counted in each brain region ranged from 35 to 453 cells with an average of 116 cells.
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11 The data was expressed as the mean value \pm S. D. (n = 4 mice). For signal intensity
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13 analyses, images of all samples were captured under the same microscopic conditions
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15 and four images per region were analyzed per mouse using the FV10-ASW software
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17 (Olympus). Briefly, cell bodies of the oligodendrocytes were outlined by means of
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19 CAII-or NG2-IR and the average fluorescent intensity of GR was measured within the
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21 outlined areas. The mean value of the sham-operated group (n = 6) in each region is
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23 considered as 100% and the relative GR intensity in the adrenalectomy group (n = 6) is
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25 shown.
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41 **Statistical analysis**

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43 Statistical analyses were performed by t-test using JMP8 software (SAS institute Japan,
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45 Tokyo, Japan) and P-values < 0.05 were considered statistically significant.
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Results

GR expression in CAII-IR cells in the gray and white matter

Expression of GRs in CAII-IR oligodendrocytes in the gray and white matter regions was examined by double immunofluorescent staining. We focused on CA1, CA3 and the dentate gyrus of the hippocampus, the primary somatosensory cortex barrel field and the basolateral amygdala in the gray matter regions and the corpus callosum, external capsule and fimbria of the hippocampus in the white matter regions. GRs were expressed broadly in various areas of the gray matter regions. The immunostaining pattern for GRs and CAII was somewhat different in each region. Since a recent paper demonstrated that different populations of oligodendrocytes are differentially distributed in various layers of the hippocampus (Vinet et al. 2010), we analyzed the differential distribution patterns of GR in oligodendrocytes in the hippocampal subregions. In the CA1 region, GRs were expressed in many cells with strong intensity, but CAII-IR cells were sparse in this region (Fig. 2). In contrast, while the number of CAII-IR cells per field was slightly greater in the CA3 and dentate gyrus regions, the intensity of GR expression was weak in these regions (Fig. 2), which was similar to the previous studies of neurons (Sarabdjitsingh et al. 2009). Most of the

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5 CAII-IR cells expressed GRs in the pyramidal cell layers of CA1 and CA3 and in the
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8 hilus of the dentate gyrus region. The expression of GRs in CAII-IR cells was observed
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11 in small nuclei with relatively strong intensity. In the primary somatosensory cortex
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14 barrel field and basolateral amygdala, most of the CAII-IR cells also expressed GRs
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17 (Fig. 2). In the basolateral amygdala, the intensity of GR immunoreactivity was weaker
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20 than that in other gray matter regions (Fig. 2). The ratio of double IR cells of GRs and
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23 CAII per total CAII-IR cells was calculated in each region. More than 80% of CAII-IR
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26 cells expressed GRs in the gray matter regions examined (Table 1). In the white matter
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29 regions, GRs were primarily expressed in small nuclei of the cells in the corpus
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32 callosum, external capsule and the fimbria of the hippocampus (Fig. 3). The number of
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35 CAII-IR cells per field was greater in the fimbria of the hippocampus compared with
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38 the corpus callosum and external capsule. More than 85% of CAII-IR cells expressed
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44 GRs in the white matter regions examined (Table 1).
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50 **GR expression in NG2-IR oligodendrocyte progenitors in the gray and white**
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53 **matter regions**
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56 Expression of GRs in NG2-IR in oligodendrocyte progenitors of the gray and
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5 white matter regions was examined by double immunofluorescent staining. We
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8 examined the same brain regions as those of CAII-IR oligodendrocytes. NG2-IR cells
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11 were observed in the pyramidal cell layers of the hippocampus, CA1 and CA3 and hilus
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13 of dentate gyrus regions in the gray matter regions (Fig. 4). In the primary
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15 somatosensory cortex barrel field and basolateral amygdala, the majority of NG2-IR
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18 cells were large cells. Almost all NG2-IR cells expressed GRs in the gray matter regions
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21 (Table 1). In the white matter regions, NG2-IR cells exhibited small nuclei in the corpus
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24 callosum, external capsule and the fimbria of the hippocampus (Fig. 5). GRs were
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27 expressed in more than 95% of NG2-IR cells (Table.1).
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37 **Absence of MR expression in the CAII-IR or NG2-IR cells in the gray and white**
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40 **matter regions**
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43 Expression of MRs in CAII-IR or NG2-IR cells in the gray and white matter
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45 regions was examined by double staining. MRs were ubiquitously expressed in CA1,
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47 CA3 and dentate gyrus in the gray matter region (Fig. 6, 7). Most of the CAII-IR or
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50 NG2-IR cells were negative for MRs in these regions. MRs were also expressed in the
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53 corpus callosum, external capsule and the fimbria of the hippocampus in the white
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5 matter region (data not shown). Similarly, very few of the CAII-IR or NG2-IR cells
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8 expressed MRs in these regions (data not shown).
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10 11 12 13 14 15 **Effects of serum CORT on GR distribution in CAII-IR and NG2-IR cells**

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18 In adrenalectomized mice, plasma levels of CORT decreased to about 20% of those
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21 in sham-operated mice (data not shown). This result indicates that plasma levels of
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24 CORT were successfully reduced by adrenalectomy. GR immunoreactivity was
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27 remarkably reduced in almost all brain regions in the adrenalectomy group. The
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30 reduction of GR immunoreactivity was also evident in both CAII-IR and NG2-IR cells
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33 in the regions examined (Fig. 8, 9). We showed the representative regions, CA1,
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36 primary somatosensory cortex, and corpus callosum of the gray and white matter
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39 regions. The relative intensity of GR immunoreactivity in CAII-IR or NG2-IR cells was
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42 significantly reduced in adrenalectomized mice compared with sham-operated mice (Fig.
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53 **Expression of GR cofactors in CAII-IR and NG2-IR cells**

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57 Expression of the GR cofactors, SRC-1 and p300, was examined in CAII-IR and
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5 NG2-IR cells. In the CAII-IR cells, neither SRC-1 nor p300 was expressed in the gray
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8 and white matter regions examined (Fig.10, 11). On the other hand, both SRC-1 and
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11 p300 were expressed in NG2-IR cells in both the gray matter (Fig. 12) and white matter
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14 regions, showing more intensely and clearly in the corpus callosum and external capsule
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17 (Fig. 13). Because NG2-IR cells express both SRC-1 and p300, we investigated
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20 colocalization of GRs with these cofactors in NG2-IR cells. We observed that
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23 co-expression of GR and p300 in NG2-IR oligodendrocyte progenitors by triple
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27 immunostaining (data not shown).
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Discussion

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Previous studies have demonstrated that GRs are expressed in various regions of the adult brain (Cintra et al. 1994; Usku et al. 2005). We confirmed that the distribution and staining pattern of GR immunoreactivity in the present study were consistent with those of previous studies using the same GR antibody (Morimoto et al. 1996). Many *in vitro* studies have demonstrated expression of GRs in cultured oligodendrocytes and that GRs are involved in promoting myelination (Vielkind et al. 1990; Chan et al. 1998). Moreover, a recent *in vivo* study of the peripheral nervous system demonstrated that Schwann cells expressed GRs and that glucocorticoids enhanced the rate of myelin formation through GRs (Morisaki et al. 2010). In the present study, we investigated GR expression in two different lineages of oligodendrocytes using immunohistochemistry. Characterization of a number of specific biochemical markers has increased our knowledge regarding the stages of oligodendrocyte maturation, both *in vitro* and *in vivo* (Baumann and Pham-Dinh. 2001). We found that GRs were expressed in mature CAII-IR oligodendrocytes in various brain regions of adult mice including both gray and white matter. These findings suggest that signal transduction through GRs in oligodendrocytes plays an important role in myelination of the adult brain *in vivo*.

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5 Furthermore, we observed that GRs were expressed in NG2-IR oligodendrocyte
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8 progenitors in various brain regions of adult mice, including both gray and white matter.
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11 Nishiyama and colleagues demonstrated that NG2-IR cells existed uniformly
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13 throughout the mature CNS, in both the gray and white matter, and that NG2-IR cells
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15 were antigenically oligodendrocyte progenitors that were differentiating into
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17 oligodendrocytes *in vivo* (Nishiyama et al. 1999). Careful examination of the
18
19 relationship between NG2-IR cells and microglia showed no overlap between the two
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21 populations of cells. Thus, NG2 should be considered as one of the most reliable and
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23 widely used markers for oligodendrocyte progenitors in the CNS (Nishiyama et al.
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25 1999; Polito et al. 2005; Kucharova et al. 2010). Our results, demonstrating GR
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27 expression in oligodendrocyte progenitors in the adult brain, suggest that GRs are
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29 involved in the differentiation processes of oligodendrocytes. These findings are
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31 consistent with the role of GRs in regulating cell growth and differentiation in the CNS
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33 (Chan et al. 1998; Alonso. 2000; Schröter et al. 2009). It has been reported that NG2-IR
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35 oligodendrocyte progenitors are also present in MS lesions (Chang et al. 2000). The
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37 reasons for the absence of remyelination in MS lesions remain unclear and it remains
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39 controversial as to whether remyelinating cells are derived from pre-existing mature
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5 oligodendrocytes and /or from proliferating progenitor cells (Wood et al. 1991;
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8 Armstrong et al. 1992). Our findings of GR expression in more than 80% of mature
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11 CAII-IR oligodendrocytes and in more than 85% of NG2-IR oligodendrocyte
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14 progenitors demonstrate that GRs could be one of the possible targets for therapies
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17 intended to enhance remyelination in MS patients.
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21 In contrast to the pattern of GR distribution in oligodendrocytes in the adult mouse
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24 brain, the other corticosteroid receptors, MRs, were not expressed in either CAII-IR
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27 cells or NG2-IR cells. In some regions of the brain, such as the hippocampal and cortex
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30 regions, GRs and MRs are co-expressed in the same neurons and exert various functions,
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33 including stress responses (Han et al. 2005). However, it remains to be determined
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36 whether or not oligodendrocytes co-express GRs and MRs. We did not observe any
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39 MR expression in either oligodendrocytes or oligodendrocyte progenitors in the adult
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42 mouse brain. These results indicate that MRs may not be involved in the myelination
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45 processes and differentiation of oligodendrocytes. In contrast, it should be noted that
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48 MRs are expressed in other types of glial cells, astrocytes and microglia after ischemic
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51 insult (Hwang et al. 2006; Oyamada et al. 2008).
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57 Next, we confirmed whether the distribution pattern of GRs in oligodendrocytes
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5 was reflective of the hormonal milieu. In the present study, adrenalectomy significantly
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8 reduced the plasma CORT level to about 20% of the basal level. Consequently, the
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11 intensity of GR expression in CAII-IR oligodendrocytes and NG2-IR oligodendrocyte
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14 progenitors was reduced significantly compared with sham-operated mice. Our finding
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17 is consistent with those of previous studies, in which there was a marked decrease of
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20 GR immunoreactivity in neurons (Ozawa et al. 1999; Usuku et al. 2005) and astrocytes
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23 (Gonzalez Deniselle MC et al. 1996), especially in the nuclear region, after
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26 adrenalectomy. These results suggest that GR expression is reflective to CORT levels in
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29 CAII-IR oligodendrocytes and NG2-IR oligodendrocyte progenitors. In the absence of
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32 CORT, GRs cannot translocate into the nucleus. However, the possibility also exists that
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35 the down-regulation of GR synthesis is induced by decreases in plasma CORT.
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40 Finally, we investigated the expression of steroid hormone cofactors in mature
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43 oligodendrocytes and oligodendrocyte progenitors of the adult mouse brain. We focused
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46 on SRC-1 and p300, because the transcriptional activity of GRs is enhanced by the
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49 recruitment of one of the transcriptional co-activators of p160 family (SRCs), with
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52 docking platforms for secondary co-activators, such as CBP, or its close homologue
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55 p300 (Fonte et al. 2007). Previous *in vitro* studies have shown that SRC-1 is a
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5 co-activator for GR in Schwann cells and astrocytes (Grenier et al. 2004 and 2006),
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8 whereas CBP/p300 is a repressor of GR in Schwann cells and in astrocytes (Fonte et al.
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11 2007). There has also been a report of the co-expression of SRC-1 and p300 in the adult
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14 rat brain (Ogawa et al. 2001). We found that there was no expression of SRC-1 or p300
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17 in mature CAII-IR oligodendrocytes. In contrast, both cofactors were expressed in
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20 NG2-IR oligodendrocyte progenitors, with more intense expression in the white matter
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23 regions, such as the corpus callosum and external capsule. These results suggest that
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27 mature oligodendrocytes in the adult mouse brain do not use SRC-1 or p300, but use
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30 some other transcriptional cofactors. Furthermore, we confirmed that p300 was
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33 co-expressed with GRs in NG2-IR oligodendrocyte progenitors, whereas we have not
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36 yet successfully accomplished the triple immunostaining of GR, SRC-1 and NG2.
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39 Although further study is needed, these results may lead to the speculation that
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42 oligodendrocytes and their progenitor cells are regulated through GRs and their
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45 cofactors in region specific manners. Future studies are necessary to elucidate what
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48 cofactors are required for GR action in CAII-IR mature oligodendrocytes.
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53 In conclusion, the present study demonstrated for the first time that GRs are
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56 expressed in different cell lineages of oligodendrocytes, i.e., the CAII-IR cell, a typical
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5 adult-type, mature oligodendrocyte, and the NG2-IR cell, an oligodendrocyte progenitor,
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8 in various brain regions of adult mice, including both gray and white matter. Using
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11 adrenalectomy in mice, we confirmed that the GRs expressed in these oligodendrocytes
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14 and oligodendrocyte progenitors are reactive to CORT. Furthermore, cofactors for GR,
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17 SRC-1 and p300, are expressed in NG2-IR oligodendrocyte progenitors, more
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20 specifically, at high levels in the white matter regions, but are not expressed in mature
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24 CAII-IR oligodendrocytes.
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26 27 28 29 30 31 **Acknowledgments**

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40 Promotion of Science to MN.
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47 **Statement of conflicts of interest**

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50 The authors report that there are no conflicts of interest.
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Figure Legends

Fig. 1: Brain regions

The shaded regions were analyzed in all experiments. Sections at 1.4-1.7 mm posterior to the Bregma according to the mouse brain atlas (Franklin and Paxinos, 2008).

For abbreviations, see Table 1.

Fig. 2: Double immunohistochemical labeling of GR and CAII in the gray matter

regions

Brain slices were double immunofluorescently stained for GR (green) and CAII (red). The staining in CA1 (a-c), CA3 (d-f), dentate gyrus (g-i), primary somatosensory cortex barrel field (j-l) and the basolateral amygdala (m-o) are shown. The cells indicated by arrows in the merged images indicate co-localization of CAII and GR (inset: magnified view). Scale bar: 50 μ m.

Fig. 3: Double immunohistochemical labeling of GR and CAII in the white matter

regions

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5 Brain slices were double immunofluorescently stained for GR (green) and CAII
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8 (red). The staining in the corpus callosum (a-c), external capsule (d-f) and fimbria of the
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11 hippocampus (g-i) are shown. The cells indicated by arrows in the merged images
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14 indicate co-localization of CAII and GR (inset: magnified view). Scale bar: 50µm.
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21 Fig. 4: Double immunohistochemical labeling of GR and NG2 in the gray matter
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24 regions
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27 Brain slices were double immunofluorescently stained for GR (green) and NG2
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30 (red). The staining in CA1 (a-c), CA3 (d-f), dentate gyrus (g-i), primary somatosensory
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33 cortex barrel field (j-l) and the basolateral amygdala (m-o) are shown. The cells
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36 indicated by arrows in the merged images indicate co-localization of GR and NG2.
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40 Scale bar: 50µm.
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47 Fig. 5: Double immunohistochemical labeling of GR and NG2 in the white matter
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50 regions
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53 Brain slices were double immunofluorescently stained for GR (green) and NG2
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56 (red). The staining in the corpus callosum (a-c), external capsule (d-f) and the fimbria of
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5 the hippocampus (g-i) are shown. The cells indicated by arrows in the merged images
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8 indicate co-localization of GR and NG2. Scale bar: 25 μ m.
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14 Fig. 6: Double immunohistochemical labeling of MR and CA II in the gray matter
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17 regions
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21 Brain slices were double immunofluorescently stained for MR (green) and CA II
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24 (red). The staining in CA1 (a-c), CA3 (d-f) and the dentate gyrus (g-i) are shown.
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27 CAII-IR cells indicated by arrowheads in the merged images do not express MRs. Scale
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30 bar: 50 μ m.
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37 Fig. 7: Double immunohistochemical labeling of MR and NG2 in the gray matter
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40 regions
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44 Brain slices were double immunofluorescently stained for MR (green) and NG2
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47 (red). The staining in CA1 (a-c), CA3 (d-f) and the dentate gyrus (g-i) are shown.
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50 NG2-IR cells indicated by arrowheads in the merged images do not express MRs. Scale
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53 bar: 50 μ m.
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5 Fig. 8: Effects of adrenalectomy on GR immunohistochemical labeling in CAII positive
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8 cells
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11 Brain slices were double immunofluorescently stained for GR (green) and CAII
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13 (red). For comparison, the staining in CA1 (a-c), primary somatosensory cortex (g-i)
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15 and the corpus callosum (m-o) after sham operation are shown. The staining in CA1
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17 (d-f), primary somatosensory cortex (j-l), and corpus callosum (p-r) after adrenalectomy
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19 are shown. GR immunoreactivity in the nucleus was very weak in the CAII-IR cells
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21 (arrows). Scale bar is 50 μ m.
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31 The relative intensity of GR-immunoreactivity in CAII-IR cells in adrenalectomized
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33 mice (n=6) compared with that in sham-operated mice (n=6) was shown (s). The
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35 intensity of GR immunoreactivity in CAII-IR cells was notably reduced in
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37 adrenalectomized mice compared with that in sham-operated mice. The difference was
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39 statistically significant (*p<0.05). Data are expressed as the mean \pm S. D.
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50 Fig. 9: Effects of adrenalectomy on GR immunohistochemical labeling in NG2 positive
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56 Brain slices were double immunofluorescently stained for GR (green) and NG2
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5 (red). For comparison, the staining in CA1 (a-c), primary somatosensory cortex (g-i)
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8 and the corpus callosum (m-o) after sham operation are shown. The staining in CA1
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11 (d-f), primary somatosensory cortex (j-l), and the corpus callosum (p-r) after
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14 adrenalectomy are shown. GR immunoreactivity in the nucleus was very weak in
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18 NG2-IR cells (arrows). Scale bar: 50 μ m.

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21 The relative intensity of GR-immunoreactivity in CAII-IR cells in adrenalectomized
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24 mice (n=6) against that in sham-operated mice (n=6) was shown (s). The intensity of
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27 GR immunoreactivity in NG2-IR cells was remarkably reduced in adrenalectomized
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30 mice compared with that in sham-operated mice. The difference was statistically
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33 significant (*p<0.05). Data are expressed as the mean \pm S.D.

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40 Fig. 10: Double immunohistochemical labeling of SRC-1 and CAII in the gray matter
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47 Brain slices were double immunofluorescently stained for SRC-1 (green) and CAII
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50 (red). The staining in CA1 (a-c), CA3 (d-f) and the dentate gyrus (g-i) are shown.
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53 CAII-IR cells indicated by arrowheads in the merged images do not express SRC-1 in
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56 these brain regions. Scale bar: 50 μ m.
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8 Fig. 11: Double immunohistochemical labeling of p300 and CAII in the gray matter
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14 Brain slices were double immunofluorescently stained for p300 (green) and CAII
15 (red). The staining in CA1 (a-c), CA3 (d-f) and dentate gyrus (g-i) are shown. CAII-IR
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17 cells indicated by arrowheads in the merged images do not express p300 in these brain
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19 regions. Scale bar: 50 μ m.
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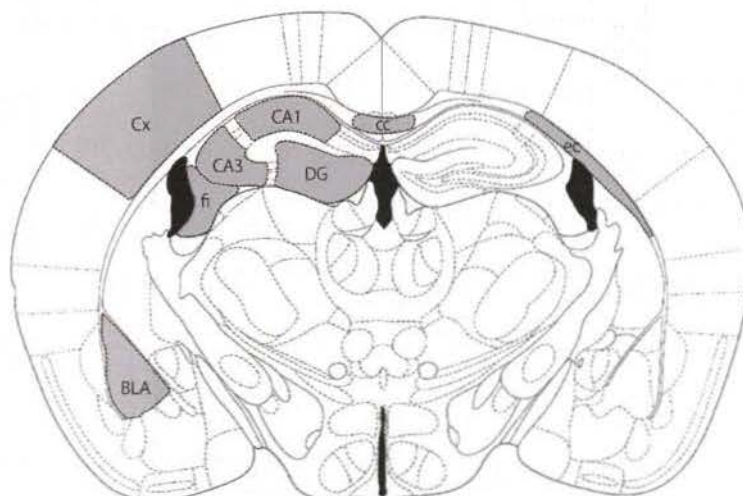
30 Fig. 12: Double immunohistochemical labeling of SRC-1 and NG2 in the gray and
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32 white matter regions
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36 Brain slices were double immunofluorescently stained for SRC-1 (green) and NG2
37 (red). The staining in CA1 (a-c), CA3 (d-f) and the dentate gyrus (g-i) in the gray matter
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39 region, and the corpus callosum (j-l) and external capsule (m-o) in the white matter
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41 region are shown. The cells indicated by arrows in the merged images indicate
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43 co-localization of SRC-1 and NG2. Expression of SRC-1 in NG2-IR cells in the white
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45 matter regions is stronger than that in the gray matter regions. Scale bar: 50 μ m.
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5 Fig. 13: Double immunohistochemical labeling of p300 and NG2 in the gray and white
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8 matter regions
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11 Brain slices were double immunofluorescently stained for p300 (green) and NG2
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13 (red). The staining in CA1 (a-c), CA3 (d-f) and the dentate gyrus (g-i) in the gray matter
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15 region, and the corpus callosum (j-l) and external capsule (m-o) in the white matter
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17 region are shown. The cells indicated by arrows in the merged images indicate
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19 co-localization of p300 and NG2. Expression of p300 was stronger in the NG2-IR cells
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27 in the white matter region compared with the gray matter region. Scale bar: 50µm.
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Figure 1

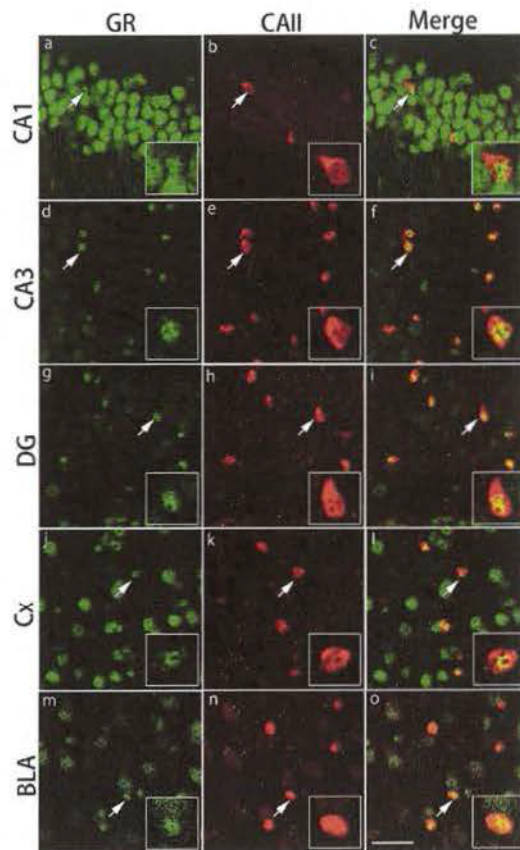


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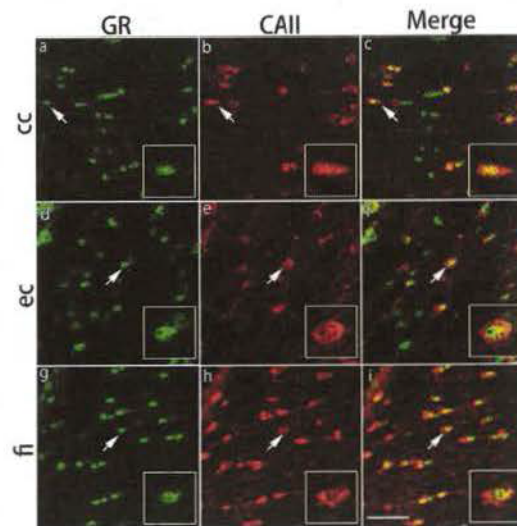
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Figure 2



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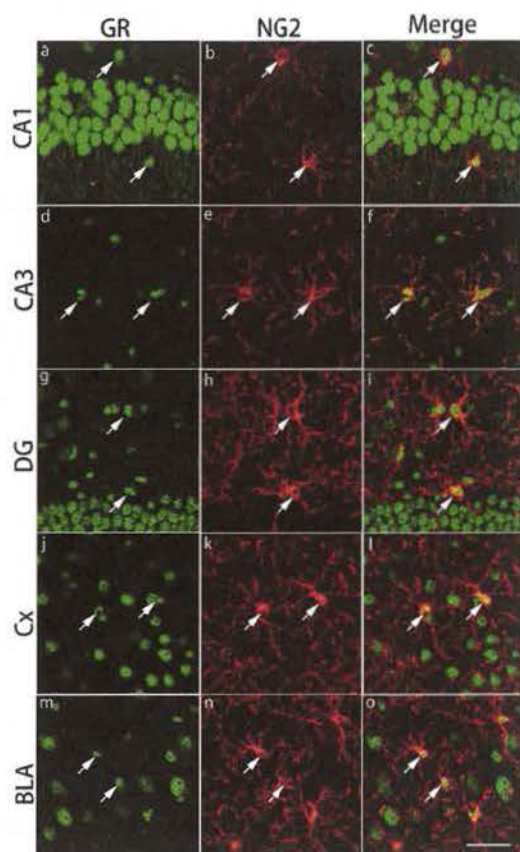
Figure 3



209x297mm (300 x 300 DPI)

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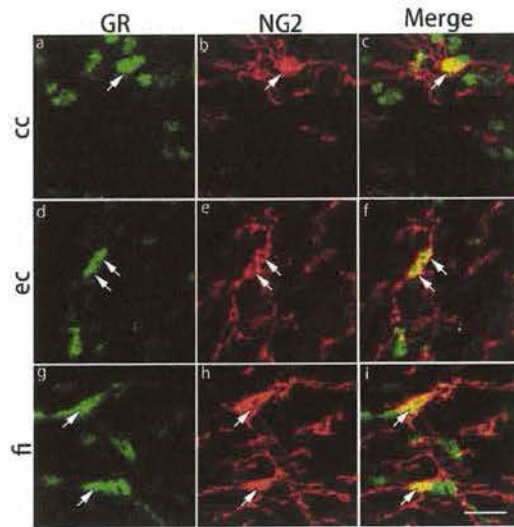
Figure 4



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Figure 5

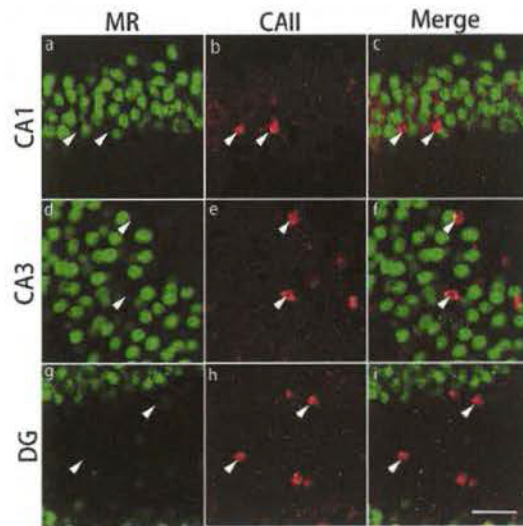


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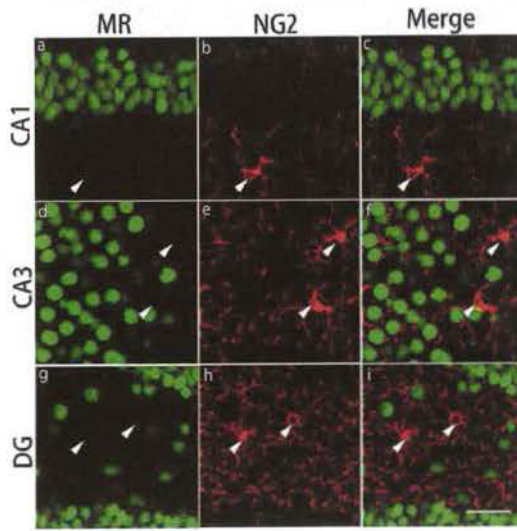
Figure 6



209x297mm (300 x 300 DPI)

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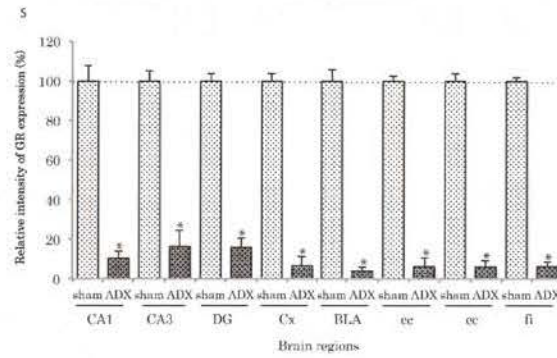
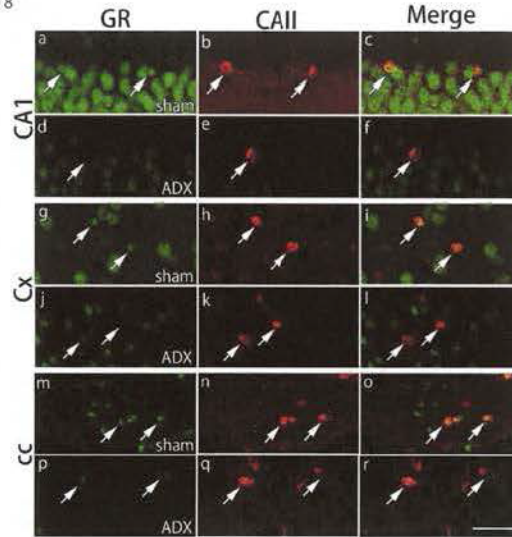
Figure 7



209x297mm (300 x 300 DPI)

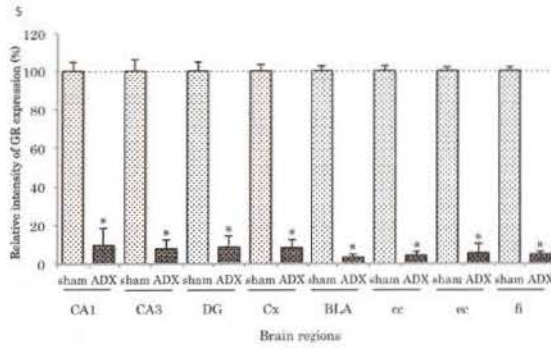
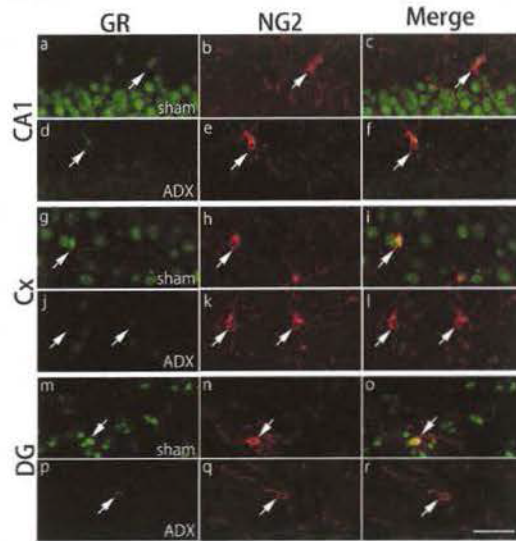
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Figure 8



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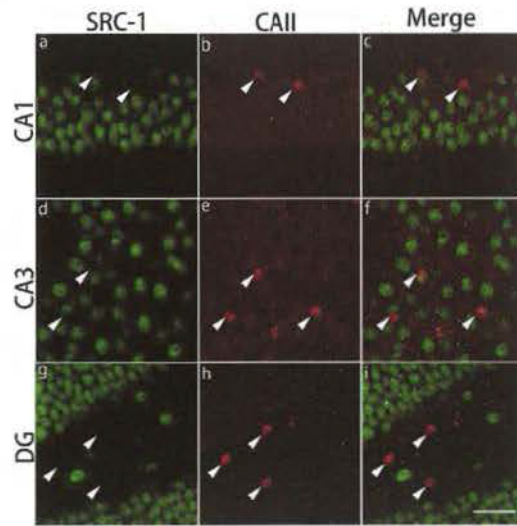
Figure 9



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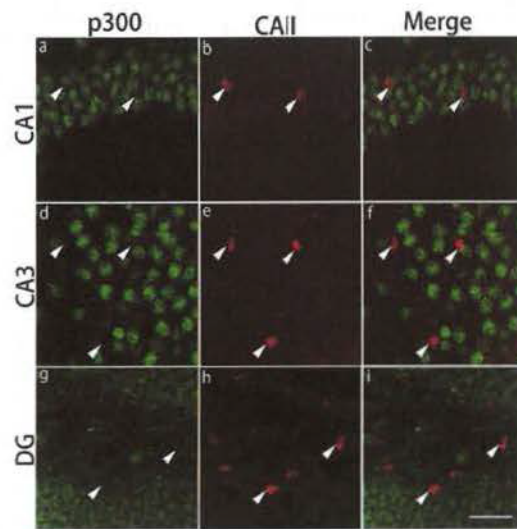
Figure 10



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Figure 11

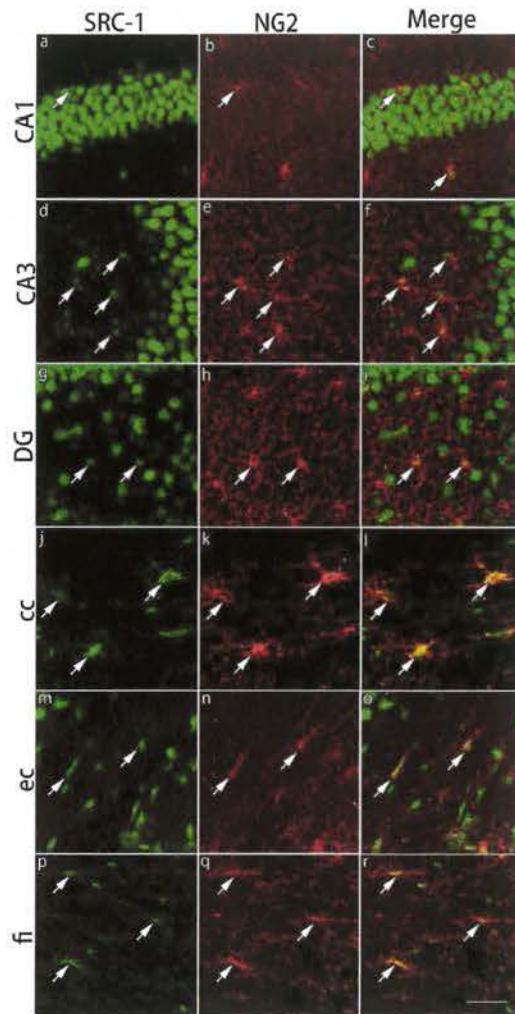


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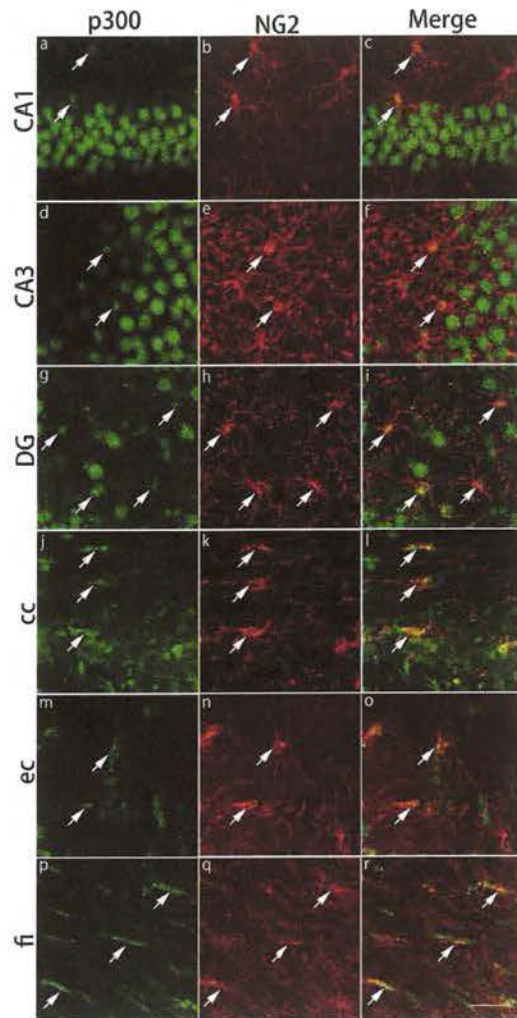
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Figure 12



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Figure 13



209x297mm (300 x 300 DPI)

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Table 1: GR expression in CAII-IR or NG2-IR cells

Brain regions	GR and CAII double IR cells per total CAII-IR cells (%)	GR and NG2 double IR cells per total NG2-IR cells (%)
Gray matter regions		
Hippocampus		
CA1	83 ± 11	100 ± 0
CA3	81 ± 5	99 ± 1
Dentate gyrus (DG)	91 ± 3	100 ± 0
Primary somatosensory cortex barrel field (Cx)	90 ± 2	99 ± 2
Basolateral amygdala (BLA)	87 ± 4	100 ± 0
White matter regions		
Corpus callosum (cc)	91 ± 1	96 ± 3
External capsule (ec)	86 ± 2	100 ± 0
Fimbria hippocampus (fi)	91 ± 2	100 ± 0

CAII-IR or NG2-IR cells in each brain region were counted in 4 mice. Data are expressed as the mean value ± S. D. (n = 4 mice).