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Glial glucocorticoid receptors in aged Fisher 344 (F344) and F344/Brown Norway rats

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Abstract

Glucocorticoid receptors (GR) regulate glial function, and changes in astrocyte gene expression are implicated in age-related pathology. We evaluated changes in astroglial GR expression in two strains of rats – Fisher 344 (F344; 4, 12 and 24 months) and F344/Brown Norway strain (F344/BN; 4, 12 and 30 months). In both strains basal levels of corticosterone were higher in the oldest groups of rats. Age-related increases in GR (+) astrocytes but not the percent of astrocytes expressing GR were observed in the hippocampus CA1 region in F344 rats. Age-related decreases in CA1 GR (+) astrocytes and the percentage of GR (+) astrocytes were observed in the F344/BN strain only. Similar strain-specific changes were observed in the dentate gyrus. In the hypothalamic paraventricular nucleus: 1) F344 rats exhibited significant decreases in the overall number of glial profiles with age, 2) F344/BN rats exhibited decreases in the numbers of GR (+) astrocytes with aging and 3) the proportion of GR (+) astrocytes decreased in older F344/BN, but not F344 rats. Overall, the data demonstrate age- and strain-related alterations in GR astrocytic expression that may explain unique phenotypic differences in brain function observed in both strains.

Keywords

glucocorticoid receptor; F344; F344/Brown Norway rat; aging; immunocytochemistry; hippocampus; hypothalamus

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1. Introduction

There are numerous unanswered questions regarding mechanisms by which glucocorticoids modulate aging. Glucocorticoids are secreted via activation of the hypothalamic-pituitary-adrenal axis (HPA axis) as part of the physiologic stress response. Glucocorticoids are the end product of this process, targeting virtually every organ system of the body, where they bind to cytoplasmic glucocorticoid receptors (GR) and translocate to the nuclei to regulate gene transcription (Reul et al. 2000). Glucocorticoids exhibit actions on the development of brain morphology, on neurochemical processes and on whole-body physiology.

One action of glucocorticoids is to regulate astroglial cell differentiation (Cotter et al. 2001). For instance, glucocorticoids modulate both astroglial motility and shape, and can inhibit glucose uptake by astrocytes (Grasso and Johnson, 1977; Freshney et al. 1980). In regulating glia in this fashion, glucocorticoids are thought to play a role in modulating neuronal-glial plasticity (Kadekaro et al. 1988).

Glial hyperplasia occurs in the aged hippocampus (Landfield et al. 1978; Sapolsky et al. 1983). Given that glucocorticoid receptors regulate glial growth and metabolism in the hippocampus (Bohn et al. 1991; Lapping et al. 1994), it is important to understand whether age-related dysfunction in glial glucocorticoid action are involved in age-related hippocampal dysfunction. A recent study gene array analysis by Blalock et al. (2003) revealed a strong correlation between age-related cognitive deficits and alterations in expression of genes associated with glial function and glial mediated neuroinflammation such as prostaglandin D synthase, complement component 1 – q and glutathione S transferase.

Past investigations examining GR expression in aging hippocampus indicate considerable variance across strains. For instance, in aged Sprague Dawley rats, a reduction of hippocampal GR binding sites has been reported (Reul et al. 1988), whereas no changes in hippocampal GR binding sites (van Eekelen et al. 1992) nor in the number of GR immunoreactive neurons in CA1 or CA2 pyramidal cell areas were observed in the aged Brown Norway rat (Cintra et al. 1994). Most studies indicate a loss of hippocampal GR mRNA, although the extent and subfield location varies with strain. Finally, glucocorticoid response element binding is increased in the hippocampus of aged Long-Evans rats (regardless of cognitive status), whereas it is decreased in aged F344/BN animals.

The present study investigates age related astroglial GR expression in the hippocampus and hypothalamus. Given the propensity for strain-related variance, we assessed astroglial GR expression in the F344 and the F344/Brown Norway F1 hybrid (F344/BN) rat strains (Masoro, 1980). The F344/BN strain appears to be a successful model of aging, as studies examining neuroendocrine responsiveness indicate a stable neuroendocrine profile late in life (Kasckow et al. 2005). On the other hand, the F344 strain exhibits greater aging-associated pathology and greater heterogeneity of glucocorticoid response with advancing age (Sprout, 1991; Segar et al. 2009), accompanied by glucocorticoid hypersecretion and altered hippocampal GR expression.

2. Materials and Methods

2.1 Animals

Male Fischer 344 rats aged 4, 12, and 24 (n = 6, 6 and 10) months and Fischer 344/Brown-Norway (F344/BN) F1 hybrid rats aged 4, 12 and 30 (n = 6, 6 and 11) months were used in this study. All animals were housed three per cage at the University of Cincinnati in a constant temperature-humidity vivarium on a 12-h light, 12-h dark cycle under the conditions of free

access to food and drinking water. All animal protocols were reviewed and approved by the institutional animal care and use committees.

2.2 Blood Sampling

Various diameter restraint cages were constructed from Plexiglas to accommodate differences in body size between the young, middle aged and old rats. A Plexiglas tab that could be moved to accommodate differences in body length was inserted at the base of the tail to further minimize movement within the restraint cage. Blood was sampled immediately after placement in restrainers. Rats were then returned to their home cages. Approximately 200–250 μ l of blood was obtained, enough to allow dual- or triple-point determinations of corticosterone (CORT).

2.3. Hormone assays

Plasma samples were collected as described above and stored at -20° C. Plasma CORT was assessed by radioimmunoassay using an MP Biomedicals, Inc. (Costa Mesa, CA) kit. Plasma ACTH concentrations were determined by radioimmunoassay using a specific antiserum generously donated by Dr. William Engeland (University of Minnesota) at a dilution of 1:210,000 and [125 I] ACTH (Amersham Biosciences, Piscataway, NJ) as labeled tracer (Jaspar and Engeland, 1991).

2.4 Perfusion and tissue processing

Animals were deeply anesthetized via an intraperitoneal injection of sodium pentobarbital (50 mg/kg) and then perfused with 100 ml 0.9% saline followed by 500 ml 4% paraformaldehyde in 0.1 M sodium phosphate buffer, pH 7.4. Brains were then removed, post-fixed overnight in the same fixative at 4° C. After fixation, brains were transferred to 30% sucrose in 0.1 M phosphate-buffered saline (PBS), pH 7.4 at 4° C until the brains sank. They were then frontally sectioned at 30 μ m-thick on a Microm freezing microtome (Richard Allen, Kalamazoo, MI). The brain sections were placed in cyroprotectant (0.1 M PBS, 30% sucrose, 1% polyvinylpyrrolidone, and 30% ethylene glycol). Cryoprotected sections were stored at -20° C until used for immunohistochemistry.

2.5 Immunohistochemistry

Fluorescence immunohistochemistry was performed using the avidin-biotin-immunoperoxidase (ABC) method. Sections from different animals and ages were all processed together. Briefly, brain sections were transferred from cyroprotectant to 50 mM potassium phosphate buffered saline (KPBS), pH 7.4. They were then rinsed in KPBS at room temperature and treated with 0.3% H_2O_2 in 50 mM KPBS for 10 minutes to inhibit the endogenous peroxidases. Sections were then treated with 50 mM KPBS, 0.1% bovine serum albumin (BSA), and 0.2% Triton X-100 for one hour at room temperature and then incubated overnight at 4° C with anti-GR rabbit polyclonal antibody (1:5000; Santa Cruz Biotechnology, Santa Cruz, CA). The following day, after washing in KPBS, they were then incubated with biotinylated secondary antibody (Vector Laboratories, Inc., Burlingame, CA), diluted 1:500 in 50 mM KPBS + 0.1% (bovine serum albumin) BSA at room temperature for one hour. After washing, sections were treated with the avidin-biotin complex (ABC Kit; Vector Laboratories, Inc., Burlingame, CA) diluted 1:500 in 50 mM KPBS + 0.1% BSA at room temperature for one hour followed by amplification of the GR protein signal by incubating sections for 10 minutes in biotinylated tyramide (TSA Biotin System; PerkinElmer Life Sciences, Boston, MA) followed by further washing and incubation in the dark for 30 minutes with Cy3-conjugated streptavidin (1:200; Jackson ImmunoResearch Laboratories, West Grove, PA).

The sections were then incubated at 4° C overnight with anti-GFAP rabbit polyclonal antibody (1:1000; DakoCytomation, Carpinteria, CA) to immunostain astrocytes. After washing

sections they were incubated for 30 minutes in Alexa 488-conjugated donkey anti-rabbit antibody (1:200; Molecular Probes, Eugene, OR). After a final wash, sections were mounted onto gelatin-coated glass slides (Gold Seal, Portsmouth, NH), air-dried overnight, and then cover slipped by 2.5% 1,4-diazobicyclo-[2,2,2]-octane, 10% polyvinyl alcohol, 5% glycerol and 100 mM Tris buffer (pH 8.5). Fluorescent labeling was visualized and photographed under a confocal microscope (Zeiss, Carl Zeiss, Inc., Thornwood, NY).

2. 6 Data analysis

Sections of brain including unilateral CA1 and dentate gyrus as well as bilateral medial parvocellular paraventricular nucleus were randomly selected from each rat. Figures 1 a and b depict the areas sampled. Confocal imaging was performed on a Zeiss 510 microscope system. All confocal images performed for analysis were collected using a 63x oil immersion lens with a numerical aperture of 0.8; z-step, 0.45 μ m; and image size 1024 $\times$ 1024 pixels. The medial area of the hippocampal CA1, dentate gyrus and medial parvocellular paraventricular nucleus were selected and 45 z-stacks per section were collected. Cy3 was excited using the red line (543 nm) and Alexa 488 was using the green line (488). All GFAP and GR expressing cells were counted in the selected region. To ensure that an activated expressing cell was not counted more than once or missed, the images were examined by scanning back and forth through the z-axis several times.

All data were analyzed using two-way ANOVA with either Fisher's protected least significant difference (PLSD) or Student Newman Keuls *post hoc* test to distinguish differences among groups. In some ANOVAs, data were transformed using a square root or log transformation to normalize the distribution.

3. Results

3.1 Cortisol Levels

The Table depicts age- and strain-related differences of corticosterone levels. The data were log transformed to make the distribution normal and one of the older F344/BN older rat values was an outlier and thus eliminated. There were significant main effects of age ($F = 13.0$; $df = 2, 38$; $p < 0.01$) but not strain ($F = 0.0032$, $df = 1, 38$, $p = 0.955$); nor was there an age by strain interaction ($F = 1.033$; $df = 2, 38$; $p < 0.366$). The CORT levels in the 24 month old F344 rats were significantly higher than CORT levels in the 12- and 4 month old F344 rats (p 's < 0.01 as per Student Newman Keuls). In addition, the CORT levels in the 30 month old F344/BN rats were significantly higher than CORT levels in the 4 month old F344 rats (p 's < 0.05 as per Student Newman Keuls).

3. 2 CA1 subfield of hippocampus

Fig. 2 depicts representative confocal micrographs from both strains of rats at varying ages in the CA1 subfields of the hippocampus (4, 12 and 24 month old F344 rats and 4, 12 and 30 month old F344/BN rats). Dual immunofluorescence staining demonstrates that GR- and GFAP-immunoreactivity (ir) are colocalized in the CA1 subfield of the hippocampus at all ages in both strains.

Figure 3 depicts age-related increases of GFAP-ir in the CA1 subfield of the hippocampus in both strains. There were significant main effects of age ($F = 36.0$, $df = 2, = 39$, $p < 0.01$) but not strain ($F = 2.33$; $df = 1, 39$; $p = 0.135$) and there was an age by strain interaction ($F = 7.44$, $df = 2, 39$; $p < 0.01$). As depicted graphically in Figure 3a, the numbers of glial profiles were significantly increased at 12 months and 24 months in the F344 rats relative to 4 month old F344 rats (both p 's < 0.01 as per Fisher's PLSD). As depicted in Figure 3a, numbers of glial profiles were also significantly increased at 30 months in the F344/BN rats relative to 4 and

12 month old rats (for 30 month vs 4 month, $p < 0.01$ and for 12 month vs 4 month, $p < 0.05$, Fisher's PLSD). Furthermore, there were significant differences in GFAP profiles between 30 mo old and 12 mo old F344/BN rats ($p < 0.05$, Fisher's PLSD).

Age-related increases in astrocytic GR in the CA1 hippocampal subfield are depicted graphically in Figure 3b. There were significant main effects of age ($F = 4.51$; $df = 2, 39$; $p < 0.05$), but not strain ($F = 25.62$; $df = 1, 39$; $p < 0.01$) and there was a significant age by strain interaction ($F = 18.86$; $df = 2, 39$; $p < 0.01$). Based on Fisher's PLSD, in the F344 rats, significant increases were observable in both 24 and 12 month old F344 rats relative to younger rats (both p 's < 0.01). In contrast, colocalized GR and GFAP-ir in F344/BN rats was decreased in 30-month and 12-month-old vs 4-month-old F344/BN rats (both p 's < 0.01 as per Fisher's PLSD).

Figure 3c graphically depicts age and strain related changes in the proportion of astrocytes expressing GR. There were significant main effects of age ($F = 14.59$; $df = 2, 39$; $p < 0.01$) and strain ($F = 16.31$; $df = 1, 39$; $p < 0.01$) as well as a significant strain by age interaction ($F = 18.75$, $df = 2, 39$; $p < 0.01$). There were age-related decreases in the proportion of astrocytes expressing GR in aged 30-month-old F344/BN rats relative to either middle-aged (12-month-old) or young (4-month-old) F344/BN rats (p 's < 0.01 , Fisher's PLSD). In F344 rats there were no age-related differences in the proportion of astrocytes expressing GR.

3. 3 Dentate Gyrus of Hippocampus

Double immunohistochemical staining clearly showed that GR- and GFAP-immunoreactivity (ir) were colocalized in the dentate gyrus subfield of hippocampus in both F344 and F344/BN strains. Figure 4 depicts representative confocal micrographs from both strains of rats at varying ages in the dentate gyrus subfields of the hippocampus (4, 12 and 24 month old F344 rats and 4, 12 and 30 month old F344/BN rats).

Both F344 and F344/BN rats exhibited age-related increases in astrocytic profiles in the dentate gyrus. There were significant main effects of age ($F = 15.72$; $df = 2, 39$; $p < 0.01$) but not strain ($F = 2.83$; $df = 1, 39$; $p = 0.101$) and there was a significant strain by age interaction ($F = 25.65$; $df = 2, 39$; $p < 0.05$). As depicted in Figure 5a, both 24 and 12 month old F344 rats exhibited significant increases relative to 4 month old rats (both p 's < 0.01 , as per Fisher PLSD). In F344/BN rats, the number of astrocytes also decreased in 30-month-old relative to both 12-month-old ($p < 0.05$, Fisher PLSD) and 4-month-old F344/BN rats ($P < 0.01$, Fisher PLSD; Figure 5a).

With regards to astrocytes expressing GR in dentate gyrus, there were significant main effects with regards to age ($F = 5.63$; $df = 2, 39$; $p < 0.01$), but not with regards to strain ($F = 3.21$; $df = 1, 39$; $p = 0.081$) and there was a significant age by strain interaction ($F = 11.52$; $df = 2, 39$; $p < 0.01$). F344 rats exhibited age-related increases of astrocytes expressing GR in the dentate gyrus; 24 month old and 12-month-old rats exhibited increases relative to 4-month-old rats (both p 's < 0.01 , Fisher PLSD; see Fig. 5b). Furthermore, 30-month-old F344/BN rats exhibited significant decreases in dentate gyrus GR expressing astrocytes relative to both 12- and 4-month-old F344/BN rats (both p 's < 0.01 , Fisher PLSD; see Figure 5b).

With regards to the proportion of astrocytes expressing GR in the dentate gyrus, there were significant main effects with regards to age ($F = 25.31$, $df = 2, 39$, $p < 0.01$), but not with regards to strain ($F = 0.280$, $df = 1, 39$, $p = 0.600$) and there was a significant age by strain interaction ($F = 35.94$, $df = 2, 39$, $p < 0.01$). The proportion of astrocytes expressing GR in the dentate gyrus was not altered in F344 rats at all three ages examined; however, with increased age, the proportion was significantly decreased in F344/BN rats. Specifically, in the F344/BN rats, the proportion in the dentate gyrus decreased in 30 month old rats, relative to 12- and 4-month-old rats (both p 's < 0.01 , Fisher PLSD; see Figure 5c).

3.4 Parvocellular Subfield of the Paraventricular Nucleus

Double immunohistochemical staining revealed that both GR- and GFAP-immunoreactivity (ir) were colocalized in the parvocellular subfield of the paraventricular nucleus in both F344 and F344/BN strains (see Figure 6). The density of GFAP-positive astrocytes in the PVN appeared more sparse than that of CA1 or DG (compare with Figure 2).

There were no significant main effects of age on the numbers of PVN astrocytic profiles ($F = 1.45$; $df = 2$; $p = 0.246$), but there were main effects of strain ($F = 5.85$; $df = 1, 39$; $p < 0.05$), with the F344 strain expressing greater overall numbers of astrocytes in this region (Figure 7A). There was no significant age by strain interaction ($F = 1.21$; $df = 2, 39$; $p = 0.309$). The GR-GFAP colocalization data were not normally distributed, requiring square-root transformation prior to analysis. Subsequent ANOVA revealed significant main effects of age ($F = 3.59$; $df = 2, 39$; $p < 0.05$) but not strain ($F = 0.319$; $df = 1, 39$; $p = 0.575$) nor was there an age by strain interaction ($F = 0.466$; $df = 2, 39$; $p = 0.631$), indicating a general loss of GR-positive astrocytes in both strains. There were significant decreases in PVN GR (+) astrocytes with aging in F344/BN rats with GR (+) astrocytes in 30-month-old F344/BN rats being lower than 12 or 4-month-old rats (all p 's < 0.05 , Fisher PLSD; see Figure 7b).

As was the case for the colocalization data, there was a significant main effects of age ($F = 5.07$; $df = 2, 39$; $p < 0.05$) but not strain ($F = 1.37$; $df = 1, 39$; $p = 0.249$) on the proportion of GR-positive GFAP staining astrocytes in the PVN. There was no significant age by strain interaction ($F = 1.89$; $df = 2, 39$; $p = 0.164$), confirming a general loss in GR positive astrocytes with age. In aged 30 month old BN/F344 rats, there were significant decreases in the percentage of astrocytes in the paraventricular nucleus which were GR (+) relative to 4- ($p < 0.05$, as per Fisher PLSD) and 12-month old rats ($p < 0.01$, Fisher PLSD; see Figure 7c). Whereas the overall effect of age is evident across strain, significant age-related changes were not present in the paraventricular nucleus of F344 rats, likely due to the high variance in this strain.

4 Discussion

4.1 Strain-dependent Glial GR Expression in Aging

Our data indicate that aging increases the total number of GFAP-positive astrocytic profiles in the hippocampal CA1 and dentate gyrus regions. These findings in the hippocampus are consistent with the well-known phenomenon of age-related gliosis and further suggest that astrocyte proliferation is a general property of the aging hippocampus, occurring within the context of both successful and unsuccessful aging.

In the CA1 hippocampal regions of the F344 rats, we found age related increases in GR (+) astrocytes, but not in the percent of astrocytes that expressed GR, indicating a parallel proliferation of GR-containing and non-GR-containing astrocytes. However, the F344/BN strain exhibited a marked age-related drop in CA1 and dentate gyrus GR (+) astrocytes and a simultaneous decrease in the percentage of astrocytes containing GR, suggesting reduced glial signaling in aged animals of this strain.

The hypothalamic PVN exhibited a markedly different aging profile than CA1 and dentate gyrus. There was no evidence for age-related gliosis in either strain and aging caused reduced GR co-localization in F344/BN strains. There is evidence for PVN GR in regulation of glucocorticoid negative feedback inhibition of glucocorticoid secretion (Di et al 2003). With our corticosterone data, both strains had elevated levels in the older animals. The cause of the specific age-related loss of GR in GFAP-positive astrocytes in the F344/BN strain is unclear. Glucocorticoids cause homologous down-regulation of GR mRNA expression (Gross and Cidlowski, 2008), suggesting that astrocytic down-regulation may be related to a history of elevated corticosterone in the F344/BN strain. However, resting corticosterone levels between

F344 and F344/BN rats do not differ significantly based on previous reports from our group (Kasckow et al 2005), suggesting an alternative mechanism. Therefore, decreased glial expression of the GR is likely mediated by other factors, including non-GR mediated inhibition of transcription and/or ubiquitin-mediated degradation in the proteosome (Wang et al, 2002).

It is generally agreed that GFAP is a reliable marker for labeling of astrocytes. There is some evidence for neural expression of GFAP in early development (Imura et al. 2003). However, all animals used in this study were of sufficient age to preclude a contribution of neural GFAP expression. Glial GFAP expression was also corroborated by morphological assessment, wherein all labeled cells had clear astrocytic morphology. It is important to note that there are populations of astrocytes that do not express GFAP (Walz, 2000). Therefore, our conclusions are limited to GR expression in GFAP-positive astrocytes. In addition, all of our cell counts were based on anatomically-matched fields in the dorsal hippocampus. We did not observe morphological evidence of marked differences between different septotemporal levels of CA1 or dentate gyrus. Nonetheless, we need to acknowledge that there may be regional variations in glial density or staining that could not be assessed by the current analysis strategy.

4.2 Astrocyte Involvement in Neuronal Function and Neurodegeneration

Astrocytes are involved in regulation of numerous neuronal functions, including energy metabolism, synapse formation and neurotransmitter transport (e.g., glutamate) (Verkhratsky et al. 1998; Shibata et al. 1997). Astrocytes also express receptor and ion channels systems known to exist in neurons (Evanko et al. 2004). Astrocytic modulation of signaling in neurons may also be mediated by the release of neuroactive compounds such as the excitatory amino acid glutamate. Transmitter release appears to be driven by a volume regulatory response triggered by hypo-osmotic conditions. The regulatory volume decrease may be mediated by any of several different pathways that increase membrane permeability; with this scenario, osmolytes travel down their concentration gradient into the extracellular space. Such signaling pathways include anion channels, hemichannels, P2X receptor channels, and transporters or multidrug resistance-proteins (Darby et al 2003; Kukley et al 2001, Stout et al 2002, Rimaniol et al 2001).

Astrocytes are also believed to play an important role in neurodegeneration. Following stroke, astrocytes can contribute to energetic endangerment of neurons by lactic acidosis. In addition, gap-junctionally connected networks of astrocytes can mediate the spread of damaging molecules to healthy neuronal cells during infarct expansion in stroke (Rossi et al. 2007). Astrocytes may also play a role in neurodegeneration through release of proinflammatory cytokines (Farfara et al 2008).

Age related changes in astrocyte GR expression could modulate neuronal function through any or all of the above mechanisms. For example, it has been proposed that glucocorticoid actions on astroglia could contribute to age related neuronal decline in certain vulnerable regions in the central nervous system (CNS; Kadekaro et al. 1988; Virgin et al. 1991) by interfering with glial glucose production, growth and glial neurotransmitter induced glycogen synthesis (Imai et al. 2001; Allaman et al. 2004; Virgin et al. 1991). Thus, the decrement in glial GR seen in F344/BN rats may have neuroprotective properties, blocking glucocorticoid-mediated activation of catabolic actions of astrocytes.

4.3 Astrocytic GR and Successful Hippocampal Aging

Successful and unsuccessful aging can be understood within the context of both pathologic and cognitive changes. At the neuropathologic level, the hippocampus expresses numerous glucocorticoid receptors and the primary structural change found during aging is synaptic loss (Nichols et al 2001). It has been reported that high levels of glucocorticoids are associated with

synaptic loss in the hippocampus, hippocampal atrophy, and cognitive decline during aging in some individuals (Lupien et al 2007) and hence “unsuccessful aging”. Proposed mechanisms to explain this have included decreased glucocorticoid response from decreased receptor levels, altered regulation of neuronal turnover in the dentate gyrus, decreased responsiveness of glial fibrillary acidic protein to glucocorticoids with aging; this latter mechanism has been proposed to facilitate reactive gliosis and loss of synapses by altering neuron-astrocyte interactions (Nichols et al 2001).

Changes in cognition are another important aspect of understanding successful aging. Studies by Hebda-Bauer et al (1999) indicate that only very old (i.e., >31 months of age) F344/BN rats perform poorly when exposed to a spatial learning task in the Morris water maze. On the other hand, F344 rats start to show cognitive problems at an earlier stage of development. Studies of Deupree et al (2003) examined various ages of F344 rats on a spatial memory task and determined that F344 rats exhibited cognitive deficits on a spatial memory task as early as 15 months of age. Moreover, unlike other strains, including the F344 rat, F344/BN do not exhibit clear evidence of age-related synapse loss in the dentate gyrus (Newton et al 2008). Thus, in terms of hippocampal structure and function, F344/BN appear to exhibit more successful aging.

Nichols et al. (2001) and O’Callaghan et al. (1991) suggest that astrocytic hypertrophy in aged hippocampus may represent a decreased responsiveness to adrenal steroids. In the F344/BN strain, age-related decreases in CA1 and dentate gyrus GR occur within the context of glial hypertrophy, consistent with this hypothesis. However, the lack of a significant change in CA1 and dentate gyrus astrocytic GR in F344 rats showing glial hypertrophy suggest that the hippocampal GR signaling mechanisms in this strain are maintained. It is possible that the persistence of the hippocampal glial GR in aged F344 rats may be associated with hippocampal pathology, as this strain is prone to age-related increases in basal as well as stress-induced glucocorticoid hypersecretion (Segar et al. 2009; Landfield et al 1978; Morano et al 1994; Hauger et al 1994). The F344 strain exhibits greater aging associated pathology along with a greater heterogeneity of HPA response (Sprout, 1991; Segar et al 2009).

Thus, the results of our study suggest an association between the loss of hippocampal astroglial GR and enhanced hippocampal and HPA axis function in the F344/BN strain. GR signaling in glia may represent an important component of the aging process, and selective down-regulation may be beneficial to maintenance of hippocampal function in senescence. Given the link between glial GR loss and successful aging, subsequent study is required to elucidate cellular mechanisms responsible for attenuated GR expression.

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Figure 1.

Schematic illustrating areas sampled in the hippocampus and paraventricular nucleus. Sampling boxes were $136 \times 136 \mu\text{m}$. Figure a depicts hippocampal areas sampled including the stratum radiatum just inferior to the CA1 pyramidal cell layer (CA1) and the hilus of the dentate gyrus (DG). Figure b depicts hypothalamic samples captured from the dorsal regions of the medial parvocellular paraventricular nucleus (PVNmpd).

Figure 2. Double-immunofluorescent images exhibited glucocorticoid receptor (GR; red) and glial fibrillary acidic protein-immunostaining (GFAP-ir; green) in the CA1 subfield of hippocampus. Colocalized GR- and GFAP-ir were observed in the F344 and F344/BN rats, with age expressed as months (mon). Scale Bar: 20 μm.

Figure 3.

These graphs represent means $\pm$ standard errors of a.) number of glial fibrillary acidic protein immunoreactive profiles (GFAP cells); b), numbers of colocalized glucocorticoid and GFAP-immunoreactive profiles (GR + GFAP Cells) and c.) the proportions of GFAP- profiles expressing GR-immunoreactivity (GR + GFAP/GFAP%) in the CA1 subfield of hippocampus in each strain. In figure a, * indicates significant differences between both 12-month-(mon) old and 24-mon-old vs 4-mon-old F344 rats ($p < 0.01$). † indicates significant differences between both 12-mon-old and 30-mon-old vs 4-mon-old F344/BN rats ($p < 0.05$ from 12-mon-old and $p < 0.01$ from 30-mon-old)** indicates significant differences between 30-mon-old vs 12-mon-old F344/BN rats ($p < 0.05$). In Fig b, * indicates significant differences with both 12-mon-old and 24-mon-old vs 4-mon-old F344 rats ($p < 0.05$). ** indicates significant differences with 30-mon-old vs both 12-mon-old and 4-mon-old F344/BN rats ($p < 0.01$). In Fig c, * indicates significant differences between 12-mon-old and 4-mon-old F344/BN rats ($p < 0.05$); ** indicates significant differences between 30-mon-old vs both 4-mon-old and 12-mon-old F344/BN rats ($p < 0.01$).

Figure 4. Double-immunofluorescent images exhibit both glucocorticoid receptor (GR; red) and glial fibrillary acidic protein-immunostaining (GFAP-ir; green) in the dentate gyrus subfield of hippocampus. Colocalized GR- and GFAP-ir were observed in the F344 and F344/BN rats, with age expressed as months (mon). Scale Bar: 20 μ m.

Figure 5.

These graphs represent means + standard errors of a.) number of glial fibrillary acidic protein immunoreactive profiles (GFAP cells); b), numbers of colocalized glucocorticoid and GFAP-immunoreactive profiles (GR + GFAP Cells) and c.) the proportions of GFAP- profiles expressing GR-immunoreactivity (GR + GFAP/GFAP%) in the dentate gyrus subfield of the hippocampus in each strain. In a, * indicates significant differences with both 12-month-old (mon) and 24-mon vs 4-mon F344 rats ($p < 0.01$ vs 12-mon and $p < 0.05$ vs 24-mon rats); ** indicates significant differences with 30-mon vs both 12-mon and 4-mon F344/BN rats ($p < 0.05$ vs 12-mon, and $P < 0.01$ vs 4-mon rats, by Fisher's PLSD test). b, * indicates significant differences with both 12-mon and 24-mon vs 4-mon F344 rats ($p < 0.05$); ** indicates significant differences with 30-mon vs both 12-mon and 4-mon F344/BN rats ($P < 0.05$ vs 12-mon, and $P < 0.01$ vs 4-mon). In c., * indicates significant differences between 12-mon vs 4-mon F344/BN rats ($p < 0.05$); ** indicates significant differences between 30-mon and both 4-mon and 12-mon F344/BN rats (both p 's < 0.01).

Figure 6. Double-immunofluorescent images exhibited glucocorticoid receptor (GR; red) and glial fibrillary acidic protein-immunostaining (GFAP-ir; green) in the parvocellular subfield of the paraventricular nucleus of the hypothalamus. Colocalized GR- and GFAP-ir were observed in the F344 and F344/BN rats with age expressed as months (mon). Scale Bar: 20 μ m.

Figure 7.

These graphs represent means $\pm$ standard errors of a.) number of glial fibrillary acidic protein immunoreactive profiles (GFAP cells); b), numbers of colocalized glucocorticoid and GFAP-immunoreactive profiles (GR + GFAP Cells) and c.) the proportion of GFAP- profiles expressing GR-immunoreactivity (GR + GFAP/GFAP%) in the parvocellular paraventricular nucleus of the hypothalamus in each strain varying with age in months (mon). In b, ** indicates significant differences between 30-mon vs both 12-mon and 4-mon F344/BN rats (both p 's < 0.05). In c, * indicates significant differences between 30-mon vs both 12-mon and 4-mon F344/BN rats (p 's < 0.05).

Table

	Corticosterone (CORT) Levels (ng/ml)	
	F344 (± Std Dev)	F344/BN* (± Std Dev)
4 mo	18.70 ± 8.15	18.97 ± 8.93
12 mo	21.50 ± 8.15	28.86 ± 8.15
24/30 mo	52.54 ± 6.31	41.17 ± 6.02

As per 2 way ANOVA, there were significant main effects of age ($F = 13.0$, $df = 2,38$; $p < 0.01$) Based on Student Newman Keuls, CORT levels were higher in 24 mo F344 rats vs 12 and 4 mo old F344 rats (p 's < 0.01); also CORT levels in 30 mo old F344/BN rats were higher than 4 mo old F344/BN rats ($p < 0.05$); Std Dev= Standard Deviation