

Review

Do glucocorticoids contribute to brain aging?

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Abstract

The hippocampus, an area with abundant glucocorticoid receptors, continues to be the focus of research on effects of glucocorticoids on the aging brain. Based on recent studies, the primary structural change found during aging is synaptic loss, rather than neuronal loss. High levels of glucocorticoids are associated with synaptic loss in the hippocampus, hippocampal atrophy, and cognitive decline during aging in some individuals. However, increasing levels of glucocorticoid are not always found since early experiences can alter sensitivity to negative feedback and the level of activation of the hypothalamic–pituitary–adrenal axis in aged individuals. New ways in which glucocorticoids may contribute to brain aging are discussed, including decreased responses to glucocorticoids possibly as a result of decreased glucocorticoid receptors and also altered regulation of neuronal turnover in the dentate gyrus. Decreased responsiveness of glial fibrillary acidic protein to glucocorticoids during aging could facilitate reactive gliosis and loss of synapses by altering neuron–astrocyte interactions. Neuronal turnover is regulated by glucocorticoids in the dentate gyrus where ongoing neurogenesis may be important for hippocampal-based memory formation in adulthood. Although the age-related decline in neurogenesis can be reversed by removal of adrenal steroids, the death of dentate granule neurons is also greatly increased by this treatment. Recent studies show age-related resistance to induced apoptosis and neurogenesis in the dentate gyrus following adrenalectomy, which is associated with increased expression of transforming growth factor- β 1. Therefore, the contribution of glucocorticoids to brain aging depends on the physiological and cellular context and some of these effects are reversible. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

Over the past decade, rapid progress has been made in elucidating molecular and cellular effects of glucocorticoids that may hold the key to understanding their role in structural and functional changes that occur during brain aging. Endogenous glucocorticoids are produced in the adrenal gland and secreted into the circulation in a circadian manner and further elevated in response to stress and disease. They easily pass through the blood–brain barrier and across cell membranes and are retained in target tissues by binding to intracellular receptors that can act as transcription factors. In the brain, glucocorticoids bind to two types of receptors, a high affinity mineralocorticoid receptor (MR) and a lower affinity glucocorticoid receptor (GR) with affinities for endogenous glucocorticoids that differ by an order of magnitude [11]. MR and GR mediate the actions of glucocorticoids in target cells by regulating gene expression at the level of DNA-dependent RNA transcription and protein synthesis. Thus, our search for neural genes regulated by glucocorticoids focussed on changes in mRNA expression in the hippocampus, a region where both MR and GR are abundantly expressed [69].

Adverse effects of pharmacological doses of glucocorticoids have been described for neurons in the rodent and primate hippocampus [1,84]. Many studies showed that elevated glucocorticoids and stress were deleterious for hippocampal neurons, which supported the idea that exposure to stress-induced elevation of glucocorticoids over the lifetime could eventually lead to neuron loss associated with aging (reviewed in Ref. [81]). Since the hippocampus is an important site for learning and memory, neurodegeneration in this region could lead to cognitive deficits associated with human aging and age-related diseases [47,51]. However, since there is little evidence of neuronal loss in rats and humans during aging, recent studies have focussed on synaptic loss as the substrate for age-related changes in the brain [21,23,57,63].

This review will focus on new insights that have been gained into brain aging based on studies of the molecular and cellular effects of glucocorticoids in neurons and glia. Changes in MR and GR could be responsible for electrophysiological and synaptic effects of glucocorticoids during aging, leading to cognitive impairment. Decreased responsiveness to glucocorticoids may result in increased expression of glial fibrillary acidic protein (GFAP) during aging and thus contribute to age-related synaptic changes. The regulation of neuron birth by glucocorticoids occurs in the adult and aging hippocampus, and decreased neurogenesis during aging may account for certain age-related changes in learning and memory. Finally, we found that mature neurons in the aged dentate gyrus are more resistant to induced apoptosis than those in young adults. This supports other data which suggests that the aged cellular microenvironment is more neurotrophic.

2. Effects of elevated glucocorticoids

Many previous studies on aging of the brain have focused on altered neuroendocrine regulation and, in particular, on that of the hypothalamic–pituitary–adrenal (HPA) axis. Endogenous glucocorticoids vary up to 50-fold over the diurnal variation set by the circadian rhythm and are further elevated in response to stress and disease. Glucocorticoids also regulate HPA activity and their own production via negative feedback at the level of the hypothalamus and pituitary. Negative feedback regulation of the HPA axis by glucocorticoids is decreased during aging, possibly due to decreased receptors (both MR and GR) in specific target tissues, which results in increased circulating levels of glucocorticoids [67,82]. Especially during challenges and in the presence of chronic diseases, aged individuals have difficulty in restoring glucocorticoid levels to baseline (reviewed in Ref. [67]). Increased HPA axis activity during aging results in higher basal glucocorticoid levels at the nadir of the circadian rhythm in humans and in higher or prolonged glucocorticoid levels after stress or in various disease states. In support of increased exposure to glucocorticoids during aging, there is an elevation of cortisol at the nadir of the circadian rhythm in aged humans, which is associated with blunted and delayed feedback inhibition in the evening [17,94].

Based on initial studies in rodents and later in primates, Sapolsky et al. [81,82] proposed a ‘feed-forward’ cascade whereby prolonged exposure to glucocorticoids was damaging to the hippocampus resulting in loss of pyramidal neurons. In addition, it was postulated that cumulative effects of stress and stress hormones, and in particular glucocorticoids, exacerbated the effects of other insults and over the lifetime led to functional deficits, including cognitive decline. However, recent data indicate that hippocampal atrophy, synaptic loss and decreased neurogenesis correlate better with functional changes during aging, than does neuronal loss [42,45,56,88]. Table 1

Table 1
Relationship between structural and functional changes during brain aging and glucocorticoid status

Changes in aged brain	Glucocorticoid levels during aging
Glial activation	Basal
Decreased neurogenesis	Basal
Decreased MR and GR	Basal
Hippocampal atrophy	High basal and increasing
Cognitive impairment	High basal and increasing
Decreased negative feedback regulation of HPA axis	Elevated
Decreased Ca ²⁺ homeostasis	Elevated
Synaptic loss	Elevated
Hippocampal pyramidal neuron loss	Pharmacological or elevated

MR, mineralocorticoid receptor; GR, glucocorticoid receptor; HPA, hypothalamic–pituitary–adrenal.

summarizes the structural and functional changes that occur during brain aging, all of which have been attributed to elevated glucocorticoids.

2.1. The role of glucocorticoids in neuronal damage during aging

The hippocampus, a site of learning and memory, is particularly vulnerable to glucocorticoid-induced neuronal damage since it contains high concentrations of MR and GR. The hippocampus is also a site of negative feedback regulation of the HPA axis [81]. Therefore, neuronal damage could blunt negative feedback leading to a further increase in glucocorticoids and more damage as predicted by the glucocorticoid cascade hypothesis [81,82]. However, an alternate explanation is that decreased hippocampal MR and GR during aging [67] could make the hippocampus and other target tissues less sensitive to effects of glucocorticoids that are necessary for maintaining homeostasis.

In the nervous system and brain, similar to elsewhere in the body, glucocorticoid responses are diverse ranging from permissive to suppressive effects [83]. A decrease in glucocorticoid responsiveness during aging could lead to a further loss of homeostasis, especially if suppressive actions of glucocorticoids that keep natural defense mechanisms from overshooting were involved [67,83]. A decade ago, de Kloet [11] suggested that imbalances in MR- and GR-mediated effects could alter responsiveness to excitatory stimuli and result in loss of homeostatic control during aging. Increases in calcium influx and decreases in inhibitory input are associated with either chronically increased or decreased glucocorticoids and can make neurons more susceptible to neurodegeneration [12]. Therefore, receptor imbalances may be responsible for the electrophysiological and synaptic changes that are found during aging and may change adaptive actions of glucocorticoids into maladaptive ones.

The causes of neuronal damage were attributed to both metabolic and calcium-mediated effects of glucocorticoids that eventually resulted in neuron death [81]. However, neuronal counts using modern stereological techniques have shown that neuron loss in the hippocampus and neocortex of rodents, primates and humans is not correlated with functional decline during aging [63,76,78,80,93]. Instead, neuronal atrophy and synaptic loss are thought to be the major contributors to age-related structural and behavioral changes in the hippocampus [9,19,23,25,79]. More recent studies using unbiased stereological counting methods have also failed to confirm that chronic high-dose exogenous glucocorticoid administration results in neuronal loss in the primate hippocampus [43]. However, exogenous corticosterone treatment of rats produced dendritic atrophy in the CA3 region of the hippocampus [96]. Neuronal shrinkage and dendritic atrophy were also found in CA2/3 regions after 1-year-long

exposure of primates to cortisol pellets implanted in the hippocampus [84]. Therefore, neuronal damage could be due to neuron atrophy or synaptic loss secondary to electrophysiological and synaptic changes that occur in response to glucocorticoids.

Glucocorticoids have a complex role in regulation of synaptic plasticity with stress-induced elevation of endogenous glucocorticoids resulting in altered calcium buffering and reversible dendritic atrophy in the hippocampus, which could result in hippocampal atrophy and adversely affect hippocampal functioning [35,56,57]. High and rising glucocorticoid levels were associated with hippocampal atrophy in a 5–6 year longitudinal study of aged humans, but moderate and decreasing levels were not [45]. Decreased volume of the dentate gyrus is associated with impaired memory in tree shrews that were chronically stressed [74]. Hippocampal atrophy during aging could be due to dendritic atrophy or to decreased neurogenesis in the dentate gyrus in response to chronically elevated glucocorticoids [8,36,38,42,57,61]. Removal of endogenous glucocorticoids by adrenalectomy (ADX) and an enriched environment can reverse the age-related decline in neurogenesis in the rodent dentate gyrus [8,34,61]. Prenatal stress can accelerate the decline in neurogenesis during aging [42]. In addition to the amount of neurogenesis, early experiences or the environment can differentially regulate the ability of adult and aged individuals to respond to stress [56,57,59,67,81]. Consequently, some aged individuals may be more susceptible to hippocampal atrophy than others and since up-regulation of neurogenesis can occur even at advanced ages, hippocampal atrophy could even be reversible in some instances. Thus, glucocorticoid effects on hippocampal plasticity may contribute to a whole continuum of functional differences that are observed during aging.

Whereas structural and functional changes could result from over-exposure to glucocorticoids over the lifetime or chronic stress elevations of glucocorticoid, they are also associated with different basal or elevated levels of glucocorticoids in aged cohorts (Table 1). The changes that occur with basal levels of glucocorticoid, for example decreased neurogenesis, may be early changes that later give rise to the structural changes that are associated with cognitive deficits, for example synaptic loss or hippocampal atrophy [8,45,57]. Similarly, glial activation could lead to synaptic loss, which eventually leads to spatial learning impairment in rats or neuronal loss in age-related neurodegenerative diseases [67,89]. Overall, these data suggest that there may be progressive changes during aging and diseases of aging, which could be correlated with different levels of glucocorticoid.

2.2. The role of glucocorticoids in cognitive impairment during aging

The association of different glucocorticoid levels with

different age-related changes could also underlie individual differences in cognitive impairment during aging. Studies show that within cohorts of aged rats or monkeys there are individuals that are either cognitively impaired or unimpaired. Cognitively impaired and unimpaired aged rats do not exhibit neuron loss in the hippocampus [5,78,80], although previous studies showed a greater decrease in neuron density in various hippocampal cell fields in the cognitively impaired groups (reviewed in Ref. [25]). However, impaired spatial learning in aged rats was associated with electrophysiological changes implicating hippocampal plasticity as the substrate for age-related dysfunction [5].

The glucocorticoid hypothesis of brain aging was recently extended to normal human aging based on evidence of hippocampal atrophy and cognitive deficits in a subset of elderly individuals with high and increasing cortisol levels over a 5-year period of study [45,47,77]. In the same study, two other groups of individuals with moderate basal cortisol levels and either decreasing or increasing cortisol levels over time did not show significant impairments in hippocampal-dependent memory tasks [45,46]. Since the 14% reduction in hippocampal volume in the study of Lupien et al. [45] was similar to that found previously in elderly individuals with mild cognitive impairment (MCI), elevated glucocorticoids may initiate hippocampal atrophy or MCI [14,95]. Previously, hippocampal atrophy in elderly subjects was also correlated with an elevation in nadir cortisol levels [49]. Hippocampal atrophy could result from either synaptic loss or decreased neurogenesis in response to glucocorticoids (Table 1).

Recent data suggest that elderly individuals with MCI and hippocampal atrophy are at risk for Alzheimer's disease [33,95]. Higher cortisol levels and increased HPA activity are sometimes found in Alzheimer's disease patients (reviewed in Ref. [67]). Therefore, the studies of Lupien et al. [45] may be identifying a subset of individuals with high and rising cortisol and hippocampal atrophy who are on a continuum between normal aging, MCI and Alzheimer's disease. The context of disease or earlier experiences is important, since these circumstances can influence how the brain responds to stress and elevated glucocorticoids and whether memory deficits are produced during aging [42,57,60,67]. High levels of glucocorticoids are associated with synaptic loss in the hippocampus, hippocampal atrophy, and cognitive decline during aging in some individuals (Table 1). However, increasing levels of glucocorticoid are not always found since early experiences can alter sensitivity to negative feedback and the level of activation of the hypothalamic–pituitary–adrenal axis in aged individuals. In addition to predicting cognitive impairment, the rate of change in glucocorticoid levels may also be an important predictor of altered physiological responses to glucocorticoids and the change from adaptive to maladaptive effects.

3. Altered glucocorticoid responsiveness during aging

Two additional ways in which glucocorticoids may contribute to brain aging are based on decreased regulation of gene expression in glia and on regulation of neuronal turnover in the dentate gyrus. The roles of glial activation and neurogenesis in brain aging are strengthened by discoveries of their regulation by food restriction, a dietary intervention into the aging process [39,40,62,66]. Food restriction increases lifespan, delays aging changes and prevents age-related disease. Food restriction is also characterized by hyperadrenocorticism [65]. Masaro [52] has proposed that food restriction is a form of hormesis, where moderate hyperadrenocorticism activates cellular mechanisms that retard aging. These mechanisms may include changes in glial activation and neurogenesis, since food restriction can delay glial activation during aging and promote the survival of newly generated neural cells in the dentate gyrus of the hippocampus [39,53,62,67].

First, we have shown decreased responsiveness of a glial mRNA to glucocorticoids during aging, which could facilitate reactive gliosis and thus lead to synaptic changes [66]. GFAP and transforming growth factor (TGF)- β 1 were examined to determine if their responsiveness to glucocorticoids changed during aging. These mRNAs were chosen for study based on their increased expression during aging, which could be due to a release from negative regulation by glucocorticoids (Table 2) [69]. New

Table 2

Opposite effects of glucocorticoids compared with other factors that regulate glial mRNA expression

Factor	Direction of mRNA response	
	GFAP	TGF- β 1
Glucocorticoids	↓	↓
Adrenalectomy	↑	↑
Neurodegeneration/lesioning	↑	↑
Injury/inflammation ^a	↑	↑
<i>Aging</i>		
Human ^b	↑	↑
Rat ^c	↑	↑
Food restriction ^d	Delayed ↑	Unknown

GFAP, glial fibrillary acidic; TGF- β 1, transforming growth factor. Adapted, in part, from N.R. Nichols, C.E. Finch, Gene products of corticosteroid action in hippocampus, *Ann. NY Acad. Sci.* 746 (1994) 145–154, with permission from The New York Academy of Sciences.

^a Reviewed in Ref. [66].

^b GFAP mRNA in human hippocampus, frontal cortex and temporal cortex [68]; TGF- β 1 mRNA in human cerebral cortex (Nichols et al., unpublished data).

^c GFAP mRNA in hippocampus and striatum [68], dentate gyrus [7], hypothalamus [71], and cerebral cortex (Nichols et al., unpublished data); TGF- β 1 mRNA in hippocampus [66], dentate gyrus [7] and striatum (Nichols et al., unpublished data).

^d [62,71].

and old findings on the regulation of glucocorticoid receptors suggest that decreased receptors could be responsible for altered glucocorticoid responsiveness during aging.

Second, we summarize recent findings on the regulation of apoptosis and neurogenesis in the dentate gyrus during aging. Induction of these processes is linked over the lifespan of the rat, suggesting a role in neuronal turnover and regeneration [4,6,8,29,73]. Since they can also be uncoupled, this may create the potential for increased hippocampal volume or hippocampal atrophy, with the latter being found in some aged individuals. These studies have provided insight into the role of early experience, the environment, and glucocorticoids on hippocampal plasticity and cognitive impairment during aging.

3.1. GFAP responsiveness to glucocorticoids is decreased during aging

In the late 1980s we aimed to identify cellular responses to glucocorticoids in the hippocampus that might mediate their physiological effects. We cloned hippocampal mRNAs that were increased or decreased in response to high doses of corticosterone (B; 40 mg/kg) administered to ADX rats compared with ADX controls [69]. Two glial mRNAs that were under negative regulation by glucocorticoids were isolated and identified as GFAP, a marker of astrocytes, and TGF- β 1, which is expressed predominantly in microglia (Table 2). Based on increased responses of GFAP and TGF- β 1 to several types of brain lesions as well as in neurodegenerative diseases, we hypothesized that the opposite effect of glucocorticoids on their expression was an adaptive response, which prevented natural defense mechanisms from overshooting (Table 2) [69]. Accordingly, if these glial responses were involved in cellular defense mechanisms in the brain, then their regulation by glucocorticoids would be a suppressive effect, which could be important in maintenance and restoration of homeostasis [83].

An increase in GFAP expression by astrocytes is a biomarker of aging, with a dramatic increase in the hippocampus in humans after the age of 65 years [10,66]. Previously, we and others found increased expression of GFAP and TGF- β 1 mRNAs in several brain regions during aging in rodents and humans (Table 2) (reviewed in Ref. [68]). Recent studies using different cloning techniques for global analysis of gene expression have confirmed that GFAP mRNA is among the ~2% of genes that alter their expression in the brain of mice and rats during aging [31,39]. Furthermore, the increase in GFAP mRNA during aging was partially prevented in long-term caloric-restricted mice (Table 2) [39]. Although several inflammatory responses, including microglial activation factors, were also up-regulated in the mouse brain during aging, TGF- β 1 was not one of them [39]. We have recently

argued that GFAP and TGF- β 1 are markers of age-related glial activation [66]. If glucocorticoids failed to down-regulate these cellular responses after insults or during aging, then they could be overactivated and lead to pathological effects. Hence, decreased responsiveness to glucocorticoids may result in increased expression of GFAP and TGF- β 1 during aging, and contribute to age-related structural changes.

We tested this hypothesis in an endocrine ablation experiment on four age groups of virgin male Fischer 344 rats (2–3, 6–8, 16–18, and 24–26 months). Glial mRNA expression was analyzed by *in situ* hybridization across whole brain sections and specifically in the dentate gyrus of the hippocampus at the cellular level. Data and representative autoradiographs of brain sections from only the youngest and oldest group of rats are shown for comparison in Figs. 1 and 2, respectively. GFAP mRNA was increased in brain sections, but not in the dentate gyrus between 2–3 and 24–26 months in sham-operated rats. The age-related changes in GFAP mRNA on the brain sections could represent changes in astrocyte responsiveness across many regions of the brain, since we previously found that GFAP expression increased after ADX in several brain regions of the adult rat, including hippocampus, cerebral cortex, striatum, cerebellum and hypothalamus [72] and corpus callosum (Nichols et al., unpublished data). A small increase was found in the dentate gyrus between 6–8 and 24–26 months in sham-operated rats as reported in Ref. [7]. As expected, GFAP mRNA was increased in response to ADX in the dentate gyrus of all age groups. Previously, we showed that increased expression of GFAP and TGF- β 1 mRNAs in the dentate gyrus after ADX was dependent on induced apoptosis in mature granule neurons following removal of trophic adrenal steroids [6,27]. We did not find ADX-induced apoptosis or the large magnitude of change in GFAP mRNA in other regions on the brain sections that we examined. However, we have not yet determined by grain counting the changes in cellular expression of GFAP in regions other than the dentate gyrus to see whether astrocytes in different brain regions reflect the response pattern for brain sections shown in Fig. 1.

Treatment of ADX rats with B decreased GFAP mRNA in all age groups compared with ADX groups in brain sections and in the dentate gyrus (Fig. 1). However, there was a significant increase in GFAP mRNA in cells in the dentate gyrus of B-treated rats at 24–26 months compared with 2–3 months, suggesting decreased responsiveness to glucocorticoid at the cellular level in the aged rats (Fig. 1) [7]. Decreased responsiveness to B administered in the drinking water was also seen in brain sections of rats at 16–18 [66] and 24–26 months (Fig. 1) compared with that of 2–3 month rats, in spite of higher levels of plasma B in the aged rats [7]. Similar findings were reported by Maines et al. [50], who showed an age-related decline in respon-

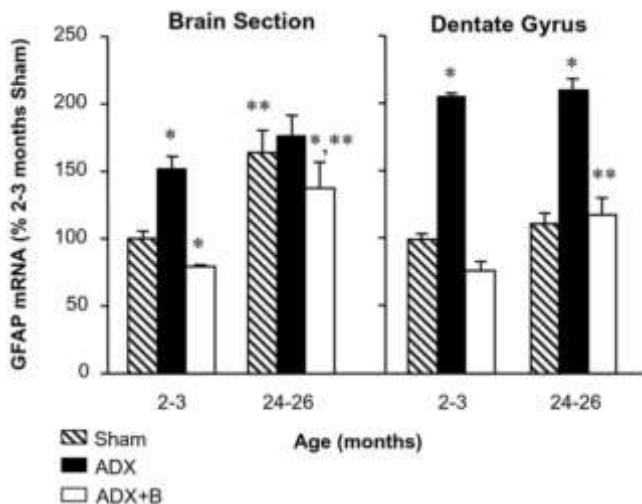


Fig. 1. Decreased glucocorticoid responsiveness of GFAP mRNA in the brain and dentate gyrus of old compared with young rats. Male Fischer 344 virgin rats at 2–3 and 24–26 months of age were compared in an endocrine ablation experiment with the following treatment groups ($n=5-13/\text{group}$): sham-operated (Sham), adrenalectomized (ADX) for 7 days, or ADX+corticosterone (B) treatment (200 $\mu\text{g}/\text{ml}$ normal saline, 0.9% NaCl) in the drinking water for 7 days. In situ hybridization was performed with radiolabeled GFAP cRNA probe on brain sections as described previously [6]. Data (mean \pm standard error) are expressed as a percentage of the 2–3 months Sham value. Statistics were performed by analysis of variance followed by Tukey post hoc tests at the 0.05 level of significance (SigmaStat software, Jandel Scientific, San Rafael, CA, USA). Digitized images of the entire brain section (left panel) for each rat were quantified from autoradiographs as optical density measurements using MCID image analysis software (Imaging Research) after subtracting film background from each section. For brain sections: $*P<0.05$, ADX vs. Sham and ADX+B groups, ADX+B vs. Sham for 2–3 months rats, ADX+B vs. ADX for 24–26 months rats; $**P<0.05$, 24–26 months vs. 2–3 months ADX+B and Sham groups. Brain section data and dentate gyrus data for 2–3 months groups are reproduced, in part, from N.R. Nichols, Glial responses to steroids as markers of brain aging, *J. Neurobiol.* 40 (1999) 585–601, ©1999 John Wiley and Sons, Inc. and reprinted by permission of Wiley–Liss, Inc., a subsidiary of John Wiley and Sons, Inc. Dentate gyrus data for 24–26 month groups are reproduced, in part, from N. Bye et al., Resistance of the dentate gyrus to induced apoptosis during ageing is associated with increases in transforming growth factor- $\beta 1$ messenger RNA, *Neuroscience*, 105 (2001) 853–862, ©2001 Elsevier Science Ltd. on behalf of IBRO and reprinted by permission. For the dentate gyrus (right panel), the average percentage cellular area occupied by grains was determined for each rat from 40 cells per rat and then for each group of rats. For the dentate gyrus: $*P<0.05$, ADX vs. Sham and ADX+B groups; $**P<0.05$, 24–26 vs. 2–3 months ADX+B groups.

siveness of GFAP to B treatment (21-day sustained release pellets) in the hippocampus of female Fischer 344 rats at 18 months, but not at 13 and 3 months of age, compared with placebo controls. There was also an age-related decline in the ability of B to down-regulate hippocampal and cortical 5HT1A receptors [50], indicating that other glucocorticoid responses are also inhibited during aging and may contribute to inability to restore homeostasis. Although GFAP is still decreased by B in the hippocampus, these data collectively suggest that the expression of

GFAP is less responsive to circulating levels of B in rats by 18 months of age.

We also showed that GFAP mRNA is not under negative regulation by glucocorticoids in aged rats since rats that were ADX for 7 days had the same expression as sham controls when analysed by in situ hybridisation on brain sections (Fig. 1) [66]. Therefore, GFAP expression is resistant to down-regulation by both endogenous and administered glucocorticoids in 24–26 month old rats. It is possible that the dentate gyrus does not show the same age-related changes due to cell turnover, since neurogenesis, gliogenesis and apoptosis occur in this region over the lifetime [4,34],

In contrast to GFAP mRNA, the age-related increase in TGF- $\beta 1$ mRNA does not appear to be due to decreased responsiveness to B. Previously, we showed that TGF- $\beta 1$ mRNA is under negative regulation by glucocorticoids [70]. TGF- $\beta 1$ mRNA is also increased in the brain during aging in activated microglia, but not in microglia with a rounded, phagocytic appearance that express MHC class II antigen (Nichols et al., unpublished data; [66]). Since microglial activation is a hallmark of brain aging, we proposed that increased expression of TGF- $\beta 1$ mRNA in activated microglia may be another glial marker of brain aging [66]. Up-regulation of TGF- $\beta 1$ mRNA during brain aging could also indicate decreased glucocorticoid responsiveness. However, in the same study shown for regulation of GFAP mRNA by glucocorticoids in Fig. 1, we did not find any evidence of altered glucocorticoid regulation of TGF- $\beta 1$ mRNA in cells of the dentate gyrus during aging [7]. Therefore, further analysis of glucocorticoid regulation of TGF- $\beta 1$ and GFAP mRNA expression in other brain regions and other regions of the hippocampus, where glial responses to neuron death are also not occurring, may reveal specific sites of altered responsiveness to glucocorticoids during aging.

3.2. Underlying mechanisms for decreased GFAP responsiveness to glucocorticoids during aging

There are several mechanisms that could account for decreased responsiveness of GFAP to regulation by glucocorticoids during aging. They include changes in MR and GR, genomic regulation at the GFAP promoter and interactions between glucocorticoids and gonadal steroids. The alteration in glucocorticoid responsiveness is likely to be mediated at the transcriptional level. Previously, we showed that transcription of GFAP mRNA is regulated by glucocorticoids [38]. In addition, the increase in hippocampal GFAP mRNA during aging represents increased transcription by middle age, which is attenuated by food restriction [37,62,97]. If the increase in GFAP transcription during aging is due to oxidative stress, secondary to microglial activation, as postulated by Morgan et al. [62], then altered responsiveness to glucocorticoids could result from oxidative damage and would be attenuated by food

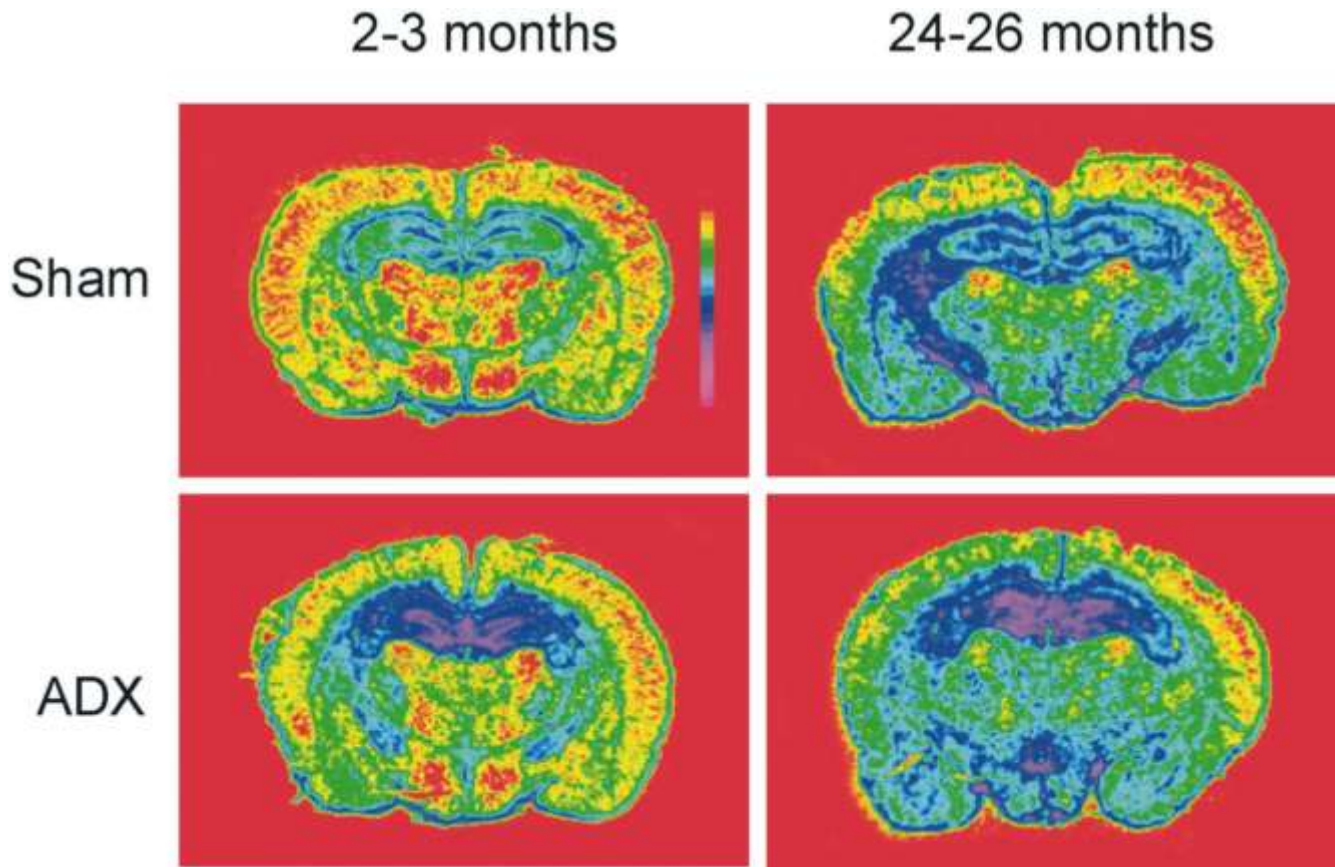


Fig. 2. Representative pseudocolor digitized images from autoradiographs of a GFAP mRNA in situ hybridization experiment. Coronal brain sections (10 microns) were hybridized with a ^{35}S -labeled antisense GFAP cRNA probe. Data are shown from young (2–3 months) and old (24–26 months) Fischer 344 male virgin rats that were Sham-operated or adrenalectomized (ADX) for 7 days. Red-orange to blue-purple on the pseudocolor scale indicates lowest to highest intensity, respectively. Note the intense purple in the dentate gyrus of the hippocampus in ADX rats, indicating up-regulation of GFAP mRNA in both age groups. Note also the higher intensities in white- (corpus callosum, fimbria, internal capsule and optic tract) and gray-matter areas (hippocampus, amygdala, thalamus, and hypothalamus) of old versus young rats in both treatment groups, which is in agreement with previous studies on old retired breeder rats that were sham-operated [66].

restriction, which reduces this type of damage. This could occur via oxidative damage to GR in astrocytes or to other transcription factors that interact with GR, including Fos, Jun and NF κ B, or to their transcriptional response elements in the GFAP promoter [38,62,97]. However, no changes in the basal expression of c-Fos or c-Jun proteins were found within the dentate gyrus in rats at 20 months compared with rats at 4 months of age and no differences were found within the aged rats with or without cognitive impairment [15].

Localized differences in responsiveness to glucocorticoids during aging could be due to tissue- or cell-specific glucocorticoid resistance secondary to specific changes in MR and GR and their signal transduction [2,13]. For example, a loss of responsiveness to glucocorticoids in the suprachiasmatic nucleus would be expected to result in a decrease in GFAP during aging. This is because glucocorticoids up-regulate GFAP in the suprachiasmatic nucleus in contrast to their effects in other brain regions, which could reflect regional differences in receptor-mediated signal

transduction as a result of neuronal–glial interactions [54,66].

Either a decrease in GR or an imbalance between MR and GR could be responsible for altered regulation of GFAP during aging. Previously, we found that the specific GR agonist, RU28362, regulated the expression of GFAP, but in the absence of specific MR agonists or antagonists, we could not rule out regulation by MR [69,72]. A decline in gonadal steroids is one factor that could lead to decreased GR and subsequently to decreased responsiveness to glucocorticoids during aging. Administration of estradiol, the active metabolite of testosterone in the central nervous system of male rats, increased glucocorticoid receptor immunoreactivity in specific regions of the hippocampus of aged rats and reversed the loss of negative feedback [18]. To assess whether a decline in gonadal steroids could be a factor in the altered GFAP regulation that we found in the 24–26 months rats shown in Fig. 1, we compared seminal vesicle weights and testosterone concentrations between young and old age groups. In Fig.

3A, we find a significant decrease in seminal vesicle to body weight ratios in the 24–26 months group that showed decreased responsiveness of GFAP mRNA to endogenous glucocorticoids (Fig. 1, Brain Section) [66]. We also found significantly lower levels of testosterone in the 24–26 months compared with 2–3 months rats (Fig. 3B). Based on these data, a possible scenario is that decreased gonadal steroids resulted in decreased glucocorticoid receptors and an increase in GFAP expression in the 24–26 months sham-operated group. However, we found no evidence of altered seminal vesicle weight [7] or decreased testosterone levels (not shown) in the 16–18 months group in this study. Thus, the resistance of GFAP and 5HT1A receptors to down-regulation by B in the 16–18 months groups in this study and that of Maines et al. [50] is likely to be due to other factors. These could include decreased MR, which occurred at 17.5 months of age and preceded changes in GR [44], or neuroinflammation and oxidative stress, which can occur by middle age [62].

It is interesting to note that food restriction, which can delay the increase in GFAP during aging as well as prevent oxidative damage (see Section 3), also decreases the GR, but not the MR, in the hippocampus and cortex of young adult rats [40]. Lee et al. [40] postulated that the decrease in GR compensates for hyperadrenocorticism and prevents damaging effects of glucocorticoids. If the GR is also decreased in food-restricted aged rats, then the data would support that a decrease in GR on its own is unlikely to be responsible for the decreased glucocorticoid responsive-

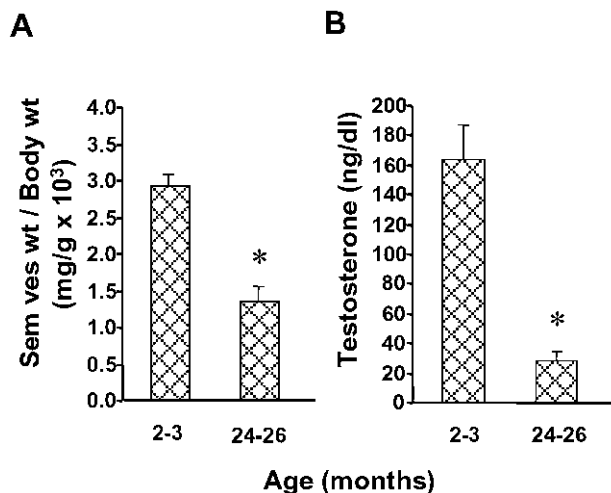


Fig. 3. Changes in seminal vesicle weight and testosterone during aging in virgin male Fischer 344 rats. (A) Seminal vesicle to body weight ratios are shown. Data are collapsed across treatment groups for each age group since there was no effect of treatment by analysis of variance: $*P < 0.001$, 24–26 months ($n = 18$) vs. 2–3 months group ($n = 31$) by *t*-test. (B) Serum testosterone concentrations in the same age groups were determined by radioimmunoassay of duplicate samples using Coat-A-Count[®] Total Testosterone kit (Diagnostic Products, Los Angeles, CA, USA) according to manufacturer's instructions. Data are also collapsed across treatment groups for comparison with seminal vesicle data: $*P < 0.001$, 24–26 months ($n = 17$) vs. 2–3 months group ($n = 26$) by *t*-test.

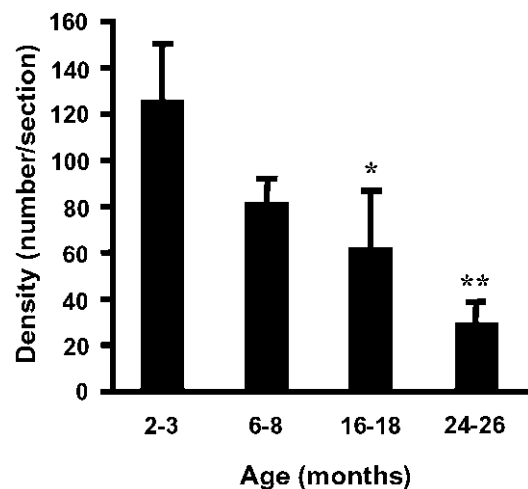


Fig. 4. Comparison of density of cells with DNA end labeling in the dentate gyrus after 7 days of ADX between different age groups of male Fischer 344 rats. The presence of DNA fragmentation in pyknotic nuclei as a cytochemical marker of apoptosis, was confirmed on brain sections using DNA end labelling in situ with digoxigenin-11-UTP by terminal transferase and detection by immunohistochemistry, as previously described [6]. The density of cells exhibiting DNA end labelling was determined by counting the number of labelled nuclei on both sides of the dentate gyrus of sections from ADX rats of each age group. Data are expressed as mean \pm standard error and statistics were performed by analysis of variance: $*P < 0.05$, 2–3 months vs. 16–18 months; $**P < 0.01$, 2–3 months vs. 24–26 months.

ness of GFAP during aging. Finally, systemic treatment with high levels of pregnenolone, the precursor of steroid hormone synthesis and a neuroactive steroid synthesized in the brain, was able to reverse the increase in GFAP during aging, but the mechanism of this effect has not yet been investigated [41]. Further studies, using animal models where age-related changes are delayed or prevented, should help to elucidate the causes of these changes during aging.

3.3. Functional consequences of increased GFAP during aging

Some of the changes that are seen in the brain during aging that have been attributed to elevated levels of glucocorticoids (Table 1) may in fact be due to decreased responsiveness to glucocorticoids. Failure to restore increased GFAP expression to basal levels could lead to prolonged glial activation and synaptic loss as a result of neuron–astrocyte interactions [66]. Due to the association of astrocytic processes with the synaptic cleft, activation of astrocytes resulted in synaptic displacement and altered effects on neurotransmission and neurosecretion influencing behavior [22,25]. A difference in perforated axonspinous synapse to neuron ratio was found between impaired and unimpaired aged rats in the absence of neuron loss by Geinisman et al. [24]. Ten years later, an increase in GFAP mRNA emerged as a predictor of age-related spatial

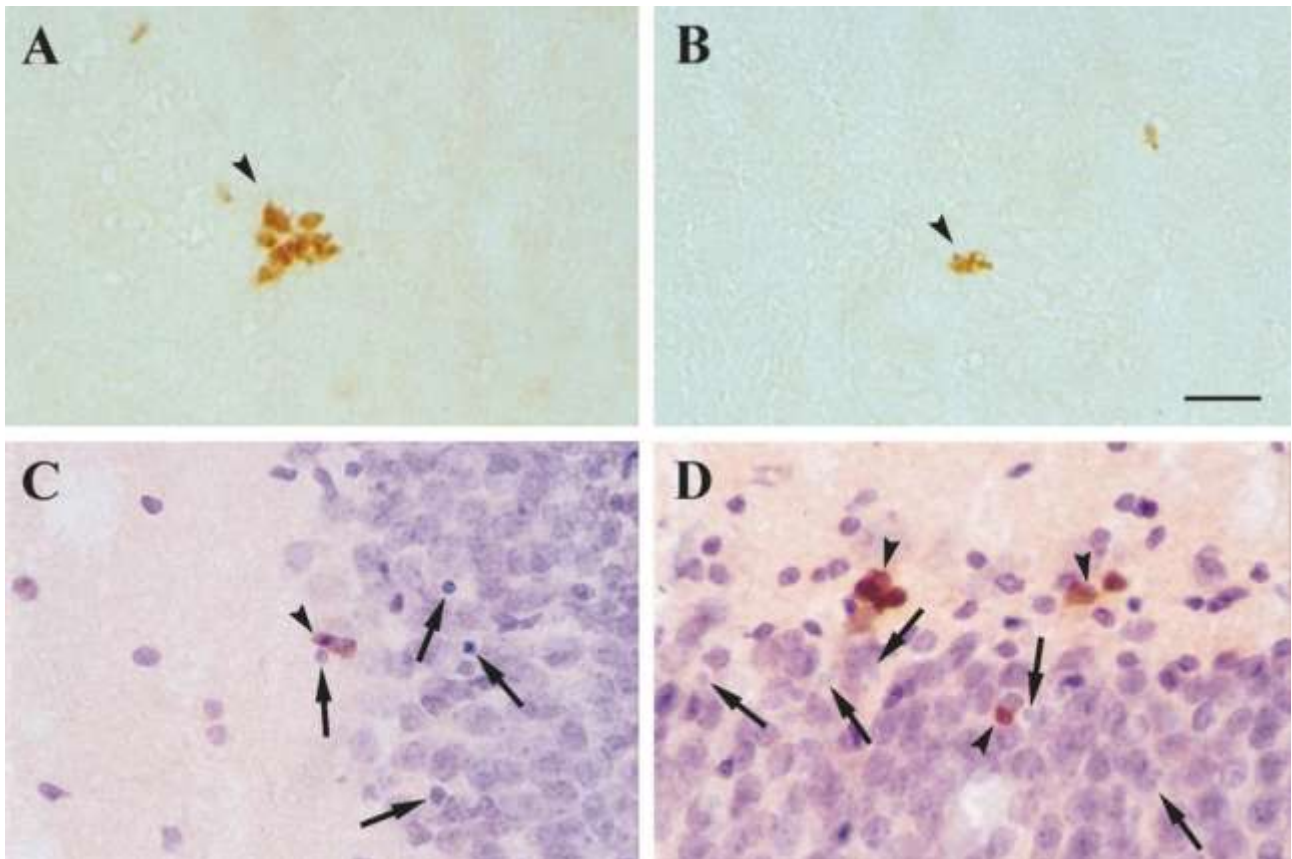


Fig. 5. Pattern of proliferating neuronal precursors in young and aged rats and comparison with location of apoptotic cells in the dentate gyrus. (A–D) Proliferating neuronal precursors were detected with an antibody against Ki67, a DNA binding protein expressed throughout most of the cell cycle [64], and visualized as a brown precipitate (arrowheads) on brain sections (30 microns). Ki67 positive nuclei are often found in clusters in 2–3 months rats (A,C,D) but are usually found as single cells (not shown) or pairs (B) in 24–26 months rats. On sections that have been counterstained with cresyl violet, atrophied apoptotic cells with pyknotic nuclei (arrows) are found in the granule cell layer, whereas most proliferating cells are found in the subgranular zone on the border between the granule cell layer and hilus. Sections were counterstained for different times to show apoptotic cells (C) or proliferating cells (D) more clearly. Scale bar is 10 microns (all frames).

learning impairment along with mRNA for manganese-dependent superoxide dismutase, a marker of oxidative stress, and beta-amyloid precursor protein [89]. In support of a direct role for GFAP and astrocytes in learning and memory, GFAP-null mice showed electrophysiological and learning impairments, including increased long-term potentiation compared with wild-type controls [55,85]. Collectively, these data suggest that GFAP is important in neuron–astrocyte interactions and specifically in modulating synaptic efficacy.

4. Resistance to adrenalectomy-induced apoptosis and neurogenesis in the dentate gyrus during aging

Recent data indicate that neuronal turnover occurs in the adult brain and may play a role in neural repair and regeneration. Neurons continue to be born in the hippocampus of birds, mammals and humans throughout their lifespan [16,27]. Spatial learning in the avian hippocampus is associated with neurogenesis and the periodic replace-

ment of old neurons with new ones [3,75]. Environmental enrichment, learning and hormonal manipulation can increase the proliferation and survival of adult-generated neurons, even in aged rats [8,20,26,30,73]. Cell death also occurs in the regions where new neurons are generated, suggesting the potential for neuron turnover for which the functional consequences are still unknown [4,86]. Neuron death by apoptosis may induce neurogenesis by release of mitogenic factors from dying cells after brain lesioning, providing a mechanism for neuron replacement [8,27,29]. The production of new neurons from neural stem cells in response to the death of mature neurons could also participate in self-repair mechanisms in the adult brain [48]. These studies make neural stem cells an attractive target for developing new ways of promoting repair and regeneration of the injured and diseased brain in adulthood.

Adult neurogenesis is also important in hippocampal-dependent learning and may be necessary for the formation of certain types of trace memories [26,87]. Glucocorticoids regulate the production of newly born granule neurons in

adult hippocampus while exerting trophic effects on mature granule neurons in the dentate gyrus [27,30]. Through these cellular responses, glucocorticoids may regulate neuronal turnover and consolidation of short- to long-term memory [30]. Furthermore, the rate of neurogenesis in the hippocampus decreases dramatically during aging and is further decreased in rats that have been exposed to prenatal stress [36,42]. Therefore, recent studies have examined the regulation of apoptosis and neurogenesis by adrenal steroids during aging. These studies have shown that ADX reverses the age-related decrease in neurogenesis and that the numbers of neurons undergoing apoptosis and neurogenesis in the dentate gyrus are correlated within individual ADX animals [8,61,73]. In addition, our data support an age-related resistance to induced apoptosis and neurogenesis following ADX.

We previously found that removal of glucocorticoids by ADX increased both apoptosis in mature granule neurons and neurogenesis in the dentate gyrus throughout the lifespan of the rat [6,73]. Fig. 4 shows that the density of nuclei with DNA fragmentation in the dentate gyrus of 7 days ADX groups decreased in 16–18 and 24–26 months rats compared with the youngest age group. We also found that the total number of neurons undergoing apoptosis in response to ADX decreased by 75% during aging [7]. Furthermore, there was an inverse relationship between the density of apoptotic cells and the expression of TGF- β 1 mRNA in the dentate gyrus across all age groups. TGF- β 1 can act as a neurotrophic factor for adult hippocampal neurons, rescuing neurons from apoptotic cell death and preventing the up-regulation of cell death proteins ([32,58]; Bye et al., unpublished data). Together, these data suggest that compensatory mechanisms in the brains of old rats may protect neurons from induced apoptosis. Such mechanisms might include increased synthesis of neurotrophic factors or lengthening of the early reversible stages of programmed cell death [90]. Changes in the timing of the phases of cell death were found in cultured sympathetic neurons after withdrawal of nerve growth factor. Older sympathetic neurons had a two times longer lag period and a two times shorter degradative phase compared with younger neurons, suggesting that they were initially more resistant to cell death, but died more quickly once they passed the commitment point [91]. Tolkovsky [90] suggested that earlier stages of cell death were prolonged in the older cells due to multiple cooperative pathways signaling for survival. In a similar manner, the increase in TGF- β 1 expression in microglia observed in the hippocampus during aging ([7,66]; Nichols et al., unpublished data) may protect granule cell neurons against induced apoptosis. It is also conceivable that inhibition of apoptosis during aging might eventually have adverse effects if the damaged cells contribute to inflammatory and neurodegenerative processes [92].

In the same cohort of rats that showed an age-related resistance to induced apoptosis after ADX (Fig. 4), we find

a parallel resistance to ADX-induced proliferation of neuronal precursors in the dentate gyrus (Figs. 5 and 6). Fig. 5 shows labeling of proliferating neuronal precursors in young and old rats at 7 days after ADX (Fig. 5A and B, respectively) as well as the spatial relationship of proliferating cells to dying cells within the dentate gyrus (Fig. 5C and D). In Fig. 6, the densities of proliferating neuronal precursors are compared in an endocrine ablation experiment across four age groups. As found in previous studies, ADX induces proliferation at all ages and the levels in the two oldest ADX groups exceed those in sham-operated 6–8 months rats (Fig. 6). However, the results in Fig. 6 also show that fewer cells are being born in all treatment groups as a function of age. Others have shown that 70–80% of the newly born cells developed into neurons in young and aged intact and ADX rats [8,42,61]. The decrease in density of proliferating cells in the dentate gyrus of ADX rats during aging has been confirmed using stereology to count the total number of proliferating cells in the same experiment [73]. The resistance of apoptosis and neurogenesis to increase by ADX suggests that, in addition to decreased neurogenesis, the capacity for turn-

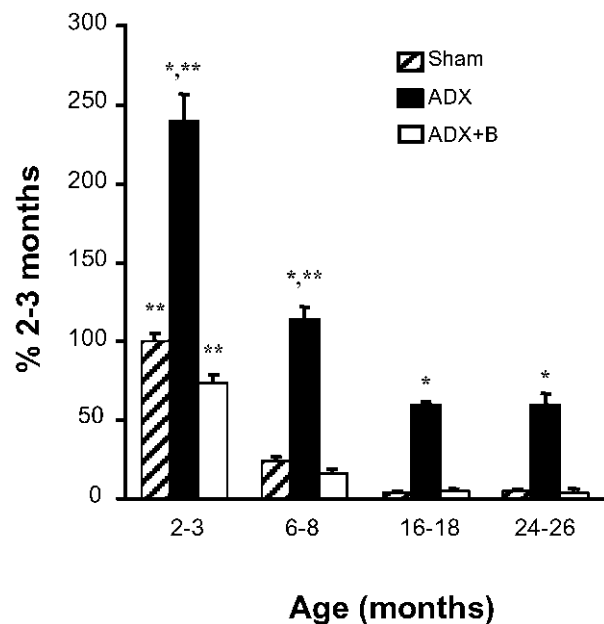


Fig. 6. Decrease in proliferating neuronal precursors in the dentate gyrus during aging. Male Fischer 344 virgin rats at 2–3, 6–8, 16–18 and 24–26 months of age are compared in the same treatment groups as described in the legend to Fig. 1. Proliferating cells were identified as described in the legend to Fig. 5. Ki67 positive nuclei were counted on four 40 \times cell fields of both sides of the dentate gyrus on three sections and averaged per section for each rat. Data (mean \pm standard error) are expressed as a percentage of the 2–3 months Sham value. Statistics were performed by analysis of variance followed by Tukey post hoc tests at the 0.05 level of significance (SigmaStat software, Jandel Scientific). There was a significant effect of treatment and age ($P<0.001$): * $P<0.05$, ADX vs. Sham and ADX+B. There was an interaction between treatment and age ($P<0.001$): ** $P<0.05$, 2–3 months versus other age groups and 6–8 vs. 16–18 and 24–26 months. Sham, sham-operated; ADX, adrenalectomized for 7 days; B, corticosterone-treated.

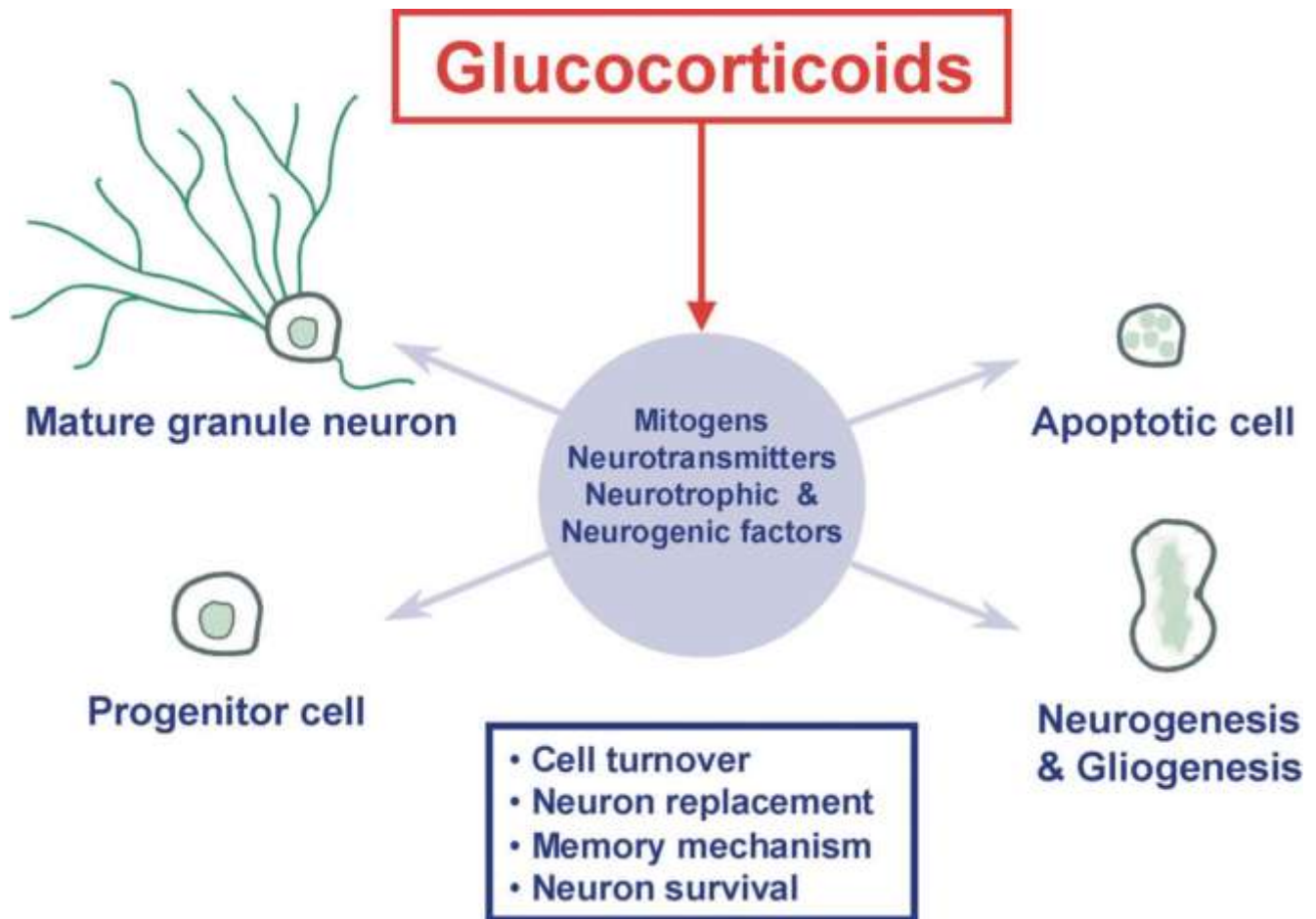


Fig. 7. Schematic diagram of the central role of glucocorticoids in regulating molecular factors which determine cellular changes in the dentate gyrus governing hippocampal plasticity and cognitive function.

over of neurons in the dentate gyrus may be reduced during aging.

Decreased neurogenesis in response to stress elevation of glucocorticoids, including prenatal stress, could underlie hippocampal atrophy and contribute to age-related cognitive decline [28,42,56,57]. Yet food restriction with its associated hyperadrenocorticism can increase neurogenesis in the dentate gyrus, which may increase synaptic plasticity and confer resistance to aging and brain injury [53]. Synaptic loss in the hippocampus during aging occurs in the molecular layer of the dentate gyrus, where decreased levels of synaptophysin staining correlated with impairment in spatial learning in aged rats [25,88]. Changes in excitatory input from the entorhinal cortex that synapse in the molecular layer can regulate neurogenesis in the dentate gyrus [27]. If a relationship between neuron replacement, changes in the hippocampal circuitry and learning is established in mammals, it could provide a novel cellular mechanism for age-related memory impairment that is not based on neuronal loss.

The hippocampus is a very plastic region of the brain, which continues to be the subject of research on how

glucocorticoids contribute to brain aging [57]. The central role of glucocorticoids in mediating relationships between cellular and molecular changes in the hippocampus is depicted in Fig. 7. Molecules that are expressed in cells of the dentate gyrus or their afferent and efferent projection sites regulate cell turnover, neuron replacement, memory formation and neuron survival. These molecules include mitogens, neurotransmitters, and neurotrophic and neurogenic factors. Future studies on the hippocampus will continue to refine our knowledge of neuronal plasticity and will identify specific cellular and molecular mechanisms that promote repair and functional recovery in the brain. Some of these may then be used to prevent synaptic loss and cognitive impairment during aging.

5. Conclusions

Based on recent studies, the primary structural change found during aging is synaptic loss, rather than neuronal loss. Findings of Lupien et al. [45] support a role for high basal and increasing cortisol levels in hippocampal atrophy

and cognitive deficits in aging humans. Recent attention on aging of the brain has focused on the physiological and cellular context of changes that result in cognitive impairment during aging, including synaptic loss, hippocampal atrophy, and decreased neurogenesis. In summary, these studies demonstrate varied responses to glucocorticoids in the hippocampus during aging depending on the physiological context, for example via interactions with other steroids, growth factors and neurotransmitters. Although glucocorticoids contribute to brain aging, they can do so either by prolonged responses to excess glucocorticoid or by decreased responsiveness to basal levels of steroid and some of these changes may be reversible.

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