Pergamon

Experimental Gerontology, Vol. 30, Nos. 3/4, pp. 229–251, 1995

0531-5565/95 $9.50 + .00

0531-5565(94)00065-4

INDIVIDUAL DIFFERENCES IN HYPOTHALAMIC–PITUITARY–ADRENAL ACTIVITY IN LATER LIFE AND HIPPOCAMPAL AGING

MICHAEL J. MEANEY, DAJAN O'DONNELL, WAYNE ROWE, BETH TANNENBAUM, ALLAN STEVERMAN, MATHIEU WALKER, N.P.V. NAIR and SONIA LUPIEN

Developmental Neuroendocrinology Laboratory and Aging Research Program, Douglas Hospital Research Center, Departments of Psychiatry and Neurology and Neurosurgery, McGill University, Montréal, Canada

Abstract—Variation in magnitude of cognitive decline in later life is a central feature of human aging. The more severe forms of dementias, such as Alzheimer's disease, clearly define one end of the spectrum. However, among those showing no obvious signs of clinical dementia there are considerable individual differences. Thus, although evidence for learning, memory, and language loss appears in some individuals as early as 50–55 years of age, many people continue to function alertly well into their 90s. These individuals exemplify what Rowe and Kahn (1987) have termed "successful" aging. The wide variability in CNS aging, often a nuisance factor in studies, are becoming a major focus for brain aging research (e.g., Gage *et al.*, 1984; Gallager and Pelleymounter, 1988; Aitken and Meaney, 1990; Issa *et al.*, 1990). Our studies over the past few years have added support to the idea that individual differences in hypothalamic–pituitary–adrenal (HPA) activity can account for part of the variation seen in neurological function among the elderly. In this article we discuss the evidence for the idea that adrenal glucocorticoids can compromise hippocampal function and, thus, produce cognitive impairments, as well as the potential mechanisms for these effects.

Key Words: glucocorticoid receptor, mineralocorticoid receptor, neuron loss, aging, hippocampus

INTRODUCTION

FUNCTIONAL DECLINE in aged mammals is associated with neurological deficits, such as neuronal dysfunction and cell death. The emphasis in our studies, then, is to understand the hormonal factors that might account for individual differences in the magnitude of aged-related neurological impairments. The studies reviewed here suggest that,

Correspondence to: Michael J. Meaney, Developmental Neuroendocrinology Laboratory, Douglas Hospital Research Center, 6875 Boul. LaSalle, Montréal (Québec) H4H 1R3, Canada.

in fact, one class of stress hormones, the adrenal glucocorticoids, which are associated with the organism's adaptational responses to stress, may potentiate neuronal dysfunction in later life. However, in addition to focusing on the amount of stress to which an animal is exposed, we must also consider individual differences in the ability to effectively control the secretion of stress hormones, and this factor seems to be critical in predicting the occurrence of some forms of age-related neuropathology in the aged rat and in explaining why certain individuals are capable of leading challenging and adventerous lives and yet sustaining a high level of functioning in later life.

The hypothalamic-pituitary-adrenal (HPA) axis

The HPA response to stress is a basic adaptive mechanism in mammals. Together with the catecholamines, the glucocorticoids secreted by the adrenal cortex account for most of the physiological responses to stimuli that threaten homeostasis. Pituitary–adrenal activity lies under the dominion of the CNS, most notably the secretion of peptidergic-releasing factors emanating from the mediobasal hypothalamus. During stress, the increased secretion of corticotropin-releasing hormone (CRH) and cosecretagogues, such as vasopressin (AVP) and oxytocin, into the portal system of the anterior pituitary enhances the synthesis and release of proopiomelanocortin-derived peptides, adrenocorticotropin (ACTH) and β-endorphin (Antoni, 1986; Rivier and Plotsky, 1986; Plotsky, 1987). Elevated ACTH levels, in turn, increase the synthesis and release of adrenal glucocorticoids. The highly catabolic glucocorticoids produce lypolysis, glycogenolysis, and protein catabolism, resulting directly or indirectly (through gluconeogenesis) in increased blood glucose levels (Munck *et al.*, 1984; Baxter and Tyrrell, 1987). These actions assist the organism during stress, in part, by increasing the availability of energy substrates at the expense of existing energy stores and of anabolic processes. However, prolonged exposure to elevated glucocorticoid levels can present a serious risk, leading to a suppression of anabolic processes, muscle atrophy, decreased sensitivity to insulin, and a risk of steroid-induced diabetes, hypertension, hyperlipidemia, hypercholesterolemia, arterial disease, amenorrhea, infertility, and the impairment of growth and tissue repair, as well as prolonged immunosupression (Munck *et al.*, 1984; Baxter and Tyrrell, 1987; Brindley and Rolland, 1989). Thus, once the stressor is terminated, it is in the animal's best interest to "turn off" the HPA stress response, and the efficacy of this process is determined by the ability of the glucocorticoids to inhibit subsequent ACTH release (i.e., glucocorticoid negative feedback).

Under resting state conditions, plasma glucocorticoid levels follow a rather pronounced circadian rhythm. In the rat, the increase in circulating titers from the nadir to the peak is about 20-fold, with peak levels occurring during the early period of the dark phase of the cycle. The increase in plasma glucocorticoid levels is derived from an increase in hypothalamic CRH release; thus, plasma ACTH levels are also increased in the PM. In addition, there is a very important increase in adrenal sensitivity to ACTH that serves to increase glucocorticoid secretion. The changes in the basal glucocorticoid signal are often unappreciated. The peak glucocorticoid levels rival those observed following stress, and the importance of the dynamic changes in the glucocorticoid signal over the cycle are only recently being studied (see Dallman *et al.*, 1993).

Circulating glucocorticoids feedback onto the pituitary and specific brain regions to inhibit the secretion of releasing factors from hypothalamic neurons and ACTH from

pituitary corticotrophs (Jones *et al.*, 1982; Keller-Wood and Dallman, 1984; Plotsky and Vale, 1984; Dallman *et al.*, 1987). In addition to these target sites, there is now considerable evidence for the importance of hippocampal inhibition of HPA activity (McEwen *et al.*, 1986; Dallman *et al.*, 1987; Jacobson and Sapolsky, 1991). The hippocampus is a major target site in the brain for glucocorticoids and a source of negative-feedback inhibition over HPA activity (reviewed in Jacobson and Sapolsky, 1991).

Likewise, there is evidence for the role of the hippocampal corticosteroid receptor systems in mediating negative-feedback inhibition over HPA activity. The brain contains two distinct receptor sites for corticosterone (the principle glucocorticoid in the rat; Reul and DeKloet, 1985, McEwen *et al.*, 1986; Funder and Sheppard, 1987; Sheppard and Funder, 1987; De Kloet, 1991). The first (type I receptor) is a mineralocorticoid site, which binds with high affinity to aldosterone, corticosterone, and the synthethic mineralocorticoids, such as RU 26752. The second (type II receptor) is a glucocorticoid site that also binds with high affinity to corticosterone and the synthetic glucocorticoids dexamethasone and RU 28362. It appears that glucocorticoid negative feedback regulation of ACTH release is mediated by both MR and GR, probably acting in synergy (see Bradbury *et al.*, 1994). The findings of studies using several different rodent models suggest that the loss of corticosteroid receptors decreases the sensitivity of the hippocampus to circulating glucocorticoids (the signal that initiates negative feedback), which, in turn, dampens negative feedback inhibition, and there ensues an increase in HPA activity and in circulating glucocorticoid levels (see Jacobson and Sapolsky, 1991).

HPA function in the aged rat

Basal levels of plasma ACTH and corticosterone have frequently been reported to be increased with age in both male and female rats (Rapaport *et al.*, 1964; Sencar-Cupovic and Milkovic, 1976; Tang and Phillips, 1978; Sapolsky *et al.*, 1983a; DeKosky *et al.*, 1984; Meaney *et al.*, 1988b, 1991, 1992; van Eekelen *et al.*, 1991; Hauger *et al.*, 1994). However, this effect has not always been found (Sonntag *et al.*, 1987), and there is considerable individual variation in HPA function among certain strains of aged rats (see below). In general, the most consistent changes occur in plasma corticosterone levels (see Sapolsky, 1991), but with the more recent discrepancies derived from studies using more rigorous sampling technics, even this conclusion is tenuous (e.g, Carnes *et al.* 1993).

In the male rat, there do not appear to be any major changes in plasma binders for corticosterone with age (Sapolsky, 1983a); however, this topic has not been extensively studied. The clearance rate for corticosterone also appears to be unchanged with age. Thus, changes in measures of total corticosterone likely reflect differences in the magnitude of the free (biologically active) corticoid signal (also see below).

In response to stress, the rat is able to mobilize a substantial increase in plasma corticosterone levels, depending upon the intensity of the stressor and the gender of the animal. In male rats, there is little age-related change in the levels of plasma corticosterone levels achieved during immobilization, restraint, novelty, or ether stress (see Sapolsky *et al.*, 1987; Meaney *et al.*, 1988b). However, the response to hemorrhage stress is substantially diminished (Hauger *et al.*, 1994). In aged female rats there is a decrease in the magnitude of the increase in plasma corticosterone levels with stress

(Hess and Reigle, 1970; Meaney *et al.*, 1991a). However, because levels of the principle glucocorticoid plasma binder, estrogen-sensitive corticosterone-binding globulin (CBG), also decrease with age in females, levels of the free, biologically active form of the steroid very likely remain constant. In summary, it seems that the corticoid signal mobilized during stress is either unaltered or decreased in the aged rat. The reason for the discrepancies here are not clear, although they may involve the nature of the stress and the neural circuitry by which the signals are transmitted to the PVN.

That which may more consistently distinguish young vs. aged rats is the difference in the duration of the elevation in plasma corticosterone following the termination of stress. In young adult animals, plasma corticosterone levels remain elevated for about 60–120 min following, for example, restraint stress. With age, there is a pronounced increase in the duration of the elevated glucocorticoid levels. At 20–26 months of age, plasma corticosterone levels in the adult male remain elevated for as long as 180–360 min following the termination of restraint stress (Sapolsky *et al.*, 1983a; Meaney *et al.*, 1988b); the duration of the corticosterone response to stress is even somewhat longer in aged females rats (Meaney *et al.*, 1991a). Thus, compared with a younger animal, the aged rat shows an impaired ability to terminate an HPA response following stress.

Hauger and colleagues (1994) have recently reported that portal concentrations of CRH are increased in the aged Fisher 344 rat under basal conditions (although these measures were derived from portal cannulations). Interestingly, they also found a decrease in pituitary sensitivity to CRF and a marked decrease in pituitary CRH receptors. A downregulation of CRH receptors could certainly occur in the presence of increased pituitary exposure to CRH. The same group also found decreased portal concentrations of AVP. In contrast, an equally recent report found almost the exact opposite pattern of changes in ACTH secretagogues; notably, decreased hypothalamic CRH in aged Fisher 344 and decreased POMC mRNA and ACTH pituitary content (Cizza *et al.*, 1994). At this point, there appears to be no clear picture of age-related changes in ACTH secretagogues.

Age-related increases in HPA activity may be associated with decreased glucocorticoid negative feedback inhibition over hypothalamic–pituitary activity, although this important point has not been studied extensively. Dilman (1981) reported that there is significantly reduced suppression of HPA function following dexamethasone administration in the aged rat. Moreover, there is a decrease in the suppression of stress-induced HPA activity by exogenous corticosterone treatment in the aged male rat (Sapolsky *et al.*, 1986b). This dampened corticoid inhibition of HPA function corresponds to a loss of brain corticosteroid receptors. In the aged rat there is a loss of both mineralocorticoid and glucocorticoid receptors for corticosterone in the hippocampus (Sapolsky *et al.*, 1983b; Ritger *et al.*, 1984; Meaney *et al.*, 1988b, 1991, 1992; Reul *et al.*, 1988), as well as a decrease in the mRNA for the glucocorticoid receptor (Peiffer *et al.*, 1991; van Eekelen *et al.*, 1991). It is interesting to note that the pattern of negative feedback impairment in the aged rat parallels the effects of hippocampal lesions (see previous section). Thus, the aged rat loses corticosteroid receptors in a brain region that is central for CNS negative feedback control over HPA activity. At this point, it appears that the loss of hippocampal corticosteroid receptors appears to underly a decrease in negative feedback control, leading to increased HPA activity under both basal and poststress conditions.

The HPA axis and hippocampal neuron loss

Hippocampal neuron loss is commonly seen in aged rats (see Coleman and Flood, 1987). Landfield *et al.* (1978, 1981) demonstrated that the occurrence of hippocampal neuron loss was positively correlated with the increase in HPA activity. They then adrenalectomized rats at midlife (12 months of age), with low-level corticosterone replacement and found that these animals showed little or no evidence of hippocampal neuron loss or the spatial memory deficits that are common in later life and closely associated with hippocampal dysfunction. Sapolsky *et al.* (1985) found that young adult rats treated for 3 months with exogenous corticosterone in the upper physiological range (mimicking the elevated basal levels seen in certain old rats) showed profound hippocampal cell loss. Moreover, the pattern of the neuron loss was strikingly similar to that observed in aged animals. This same corticosterone treatment also produces impaired spatial memory performance in midaged rats (Bodnoff *et al.*, 1995).

Glucocorticoids appear to potentiate neuron loss by rendering hippocampal neurons metabolically vulnerable. The basis for this hypothesis has come from a series of elegant studies largely from Sapolsky's group (Kadekaro *et al.*, 1986; Koide *et al.*, 1986; Sapolsky, 1990), based on the observation that glucocorticoid inhibit glucose uptake by hippocampal neurons (Horner *et al.*, 1990). Neurons have very limited energy storage capacity and a high metabolic rate (Seisjo, 1981), and are compromised by a blockade on glucose uptake. Elevated glucocorticoids increase the damage to hippocampal neurons associated with a wide range of insults, including, excitotoxins, antimetabolites (i.e., 3-acetylpyridine), oxygen radical generators, and hypoxic-ischemia. In each case, the corticoids appear to increase the vulnerability of the neurons to the toxins by decreasing the cell's metabolic capacity to survive the insult, and this effect is blocked by supplementary administration of additional energy substrates such as mannose and ketone bodies (Sapolsky, 1986).

In addition, glucocorticoids can inhibit glutamate uptake by glial cells (Virgin *et al.*, 1991), a major route for the elimination of glutamate from the synapse. Thus, elevated glucocorticoid levels enhance the increase in extracellular glutamate levels associated with kainic acid administration such that glucocorticoids can also compromise hippocampal neuron survival by enhancing the glutaminergic signal associated with a range of neurological insults. NMDA receptor antagonists reduce the endangerment of hippocampal neurons by glucocorticoids (Armanini *et al.*, 1990). Interestingly, the effects of glucocorticoids on glutamate uptake were observed only under conditions of reduced glucose availability. Because glutamate uptake is costly in terms of energy, and glucocorticoids also decrease glucose uptake by glial cells, it is possible that the effect on glial uptake of glutamate is a consequence to the glucocorticoid regulation of intracellular energy substrates. In summary, glucocorticoids, therefore, both increase the toxic glutamate signal and decrease the metabolic capacity of neurons to survive. At present, it is not clear whether the events occurring in response to acute insult are comparable to those occurring in the aged brain.

Another intriguing questions concerns the regional specificity of the hippocampal damage seen in aged rats. The loss of hippocampal neurons with age in the rat is clearly most pronounced in the pyramidal neurons of the CA1 cell field. By contrast, the granule cells of the dentate gyrus are remarkably resistant to age-related damage (and

to damage by acute neurological insult). Yet, in adult rats, both regions contain comparable levels of glucocorticoid (and mineralocorticoid) receptors and are highly glutamate sensitive. One factor that might, in part, explain the differential loss of neurons in these regions concerns the dynamic changes in glucocorticoid receptor gene expression following neurological insult. O'Donnell *et al.* (1993) found that lesions of the entorhinal cortex (ECL), which eliminate a major glutaminergic input into the hippocampus and likely result in a massive release of glutamate from dying entorhinal neurons immediately following the lesion, produce regionally specific changes in glucocorticoid receptor mRNA levels. One day following ECL lesions glucocorticoid receptor mRNA levels are significantly decreased in the dentate dyrus, an effect that could result in a loss of sensitivity to circulating glucoocorticoids. At the same time, glucocorticoid receptor mRNA levels are increased in CA1. This differential response to the insult could contribute to the loss of CA1 neurons and the survival of the dentate neurons. The question that remains is whether these findings on the acute effects of glucocorticoids following neurological insult have any bearing on the process of neuron loss during aging.

Acute effects of glucocorticoids on hippocampal function

In addition to these long-term effects on neuron survival, exposure to elevated glucocorticoid levels also seems to alter cognitive function in a short-term manner. The fact that many of these effects are reversed following the removal of the enhanced corticoid signal, suggests that they are not related to neuron loss. There are several reports (e.g., Whelan *et al.*, 1980; Starkman and Schteingart, 1981; Starkman *et al.*, 1981) of impaired cognitive function associated with elevated glucocorticoid levels of an either endogenous (e.g., Cushing's disease) or exogenous origin (glucocorticoid therapies), and the degree of impaired cognitive function is correlated with cortisol levels (Starkman *et al.*, 1981). Interestingly, these conditions are also associated with evidence (obtained using CT scans) of cerebral atrophy (Heinz *et al.*, 1977; Benston *et al.*, 1978; Okuno *et al.*, 1980). Both the impaired cognitive functioning and the cerebral atrophy are reversed following a decline in glucocorticoid levels. Finally, the magnitude of the cognitive deficits (so-called pseudodementia) associated with depression have been found to be correlated with plasma glucocorticoid levels (Rubinow *et al.*, 1984). Taken together, these reports indicate that elevated glucocorticoid levels can compromise brain function over a short term of exposure, independent of their effects on neuron loss.

It has been known for some time that glucocorticoids can decrease unit activity in the dorsal hippocampus (Pfaff *et al.*,, 1971). However, the mechanisms underlying these effects are only now becoming clarified. In a series of papers, Joels and DeKloet (1989) and Kerr *et al.* (1989) have demonstrated that in young adult rats glucocorticoids increase both the amplitude and the duration of calcium-dependent slow afterhyperpolarization (AHP) in dorsal hippocampus slice preparations. The effect is mediated by glucocorticoid receptors and the time course is consistent with that of a genomic effect. Such AHPs dampen the neurons response to subsequent excitatory inputs. Interestingly, reduced AHPs are associated with the acquisition of new information. Glucocorticoids also decrease the norephinepherine-evoked augmentation of depolarization-induced neuronal activity in CA1 neurons. Together, these effects would serve to

decrease overall excitability in hippocampal neurons. Kerr *et al.* (1989) demonstrated that the same pattern of effects is apparent in aged vs. young rats, and the difference is reversed when the aged animals are adrenalectomized.

Kerr *et al.* (1989) also provided evidence that the increased slow AHP was mediated by an increase in calcium conductance (slow AHPs are related to slow calcium-dependent potassium conductance). The effect on calcium homeostasis is of considerable interest, considering the wealth of evidence demonstrating the cytotoxic effects of elevated intracellular calcium. Indeed, there may occur a continuum of glucocorticoid effects: in the early stages, the elevated glucocorticoid signal might be associated with elevated calcium-dependent AHPs, compromising hippocampal function, and continued exposure to elevated glucocorticoid levels might then result in neuron death (also see Kerr *et al.*, 1991). Indeed, the studies from Landfield's group have shown that increased Ca influx through voltage-sensitive channels at the soma (producing increased AHPs) is a major feature of hippocampal aging in the rat (e.g., Landfield and Pitler, 1984).

The results of these studies provide clear evidence of short-term effects of glucocorticoids on hippocampal function. These findings also suggest that cognitive processes dependent on hippocampal function would be compromised in the presence of elevated glucocorticoid levels (again, independent of neuron loss). Evidence for such an effect has emerged from both in vivo and in vitro experiments using the long-term potentiation model of synaptic plasticity (an electrophysiological model for learning and memory). Elevated levels of glucocorticoids dampen LTP formation in the hippocampus in a concentration-dependent manner (Foy *et al.*, 1987; Bennet *et al.*, 1991). Adrenalectomy, by contrast, enhances LTP formation (Diamond *et al.*, 1990; submitted).

In a lower range of concentrations, corticosterone actually enhances LTP formation, resulting in an inverted-U shaped function between steroid levels and potentiation (Diamond *et al.*, 1992). Overall, low–moderate basal levels of corticosterone, which activate principally mineralocorticoid receptors (see Reul and DeKloet, 1985; Meaney *et al.*, 1988a), facilitates LTP formation, while high basal to stress levels, which activate glucocorticoid receptors, impair LTP formation. Indeed, acute stress has been shown to disrupt hippocampal LTP formation (Diamond *et al.*, 1990), and this effect appears to be mediated by both increased adrenal release of corticosterone and enkephalins (see Diamond *et al.*, 1992).

The studies of Joels and DeKloet (1989, 1990) have shown that glucocorticoid receptor activation increases the magnitude of AHP; mineralocorticoid receptor activation produces the opposite effect. Because an increase in hippocampal AHP would be expected to decrease potentiation, it is likely that this finding provides a mechanism for the reduced LTP associated with elevated glucocorticoid levels. Conversely, low corticosterone levels might facilitate LTP formation by decreasing hippocampal AHP. Indeed, Yau *et al.* (submitted) found a positive correlation between mineralocorticoid receptor mRNA expression in CA1 and spatial learning, while Oitzl and de Kloet (1992) found that the mineralocorticoid receptor antangonist, spironolactone, impaired spatial learning. Finally, Reul *et al.* (1988) found that a neurotropic agent that increases mineralocorticoid receptor binding in the hippocampus also improves memory performance. Together, these findings support the hypothesis that mineralocorticoid receptor activation enhances hippocampal LTP and spatial learning. As in the previous section on neuron survival, these findings underscore the importance of an efficient modulation

of corticosterone levels for the organism and demonstrate that glucocorticoids impair electrophysiological signal processing in the hippocampus, providing another mechanism for the impaired cognitive function associated with exposure to high corticoid levels.

Each of these studies has focused on the acute effects of elevated glucocorticoids. In the context of the aged rat, glucocorticoid levels are chronically elevated, and variations in the duration of exposure to elevated steroid levels can result in varying effects on neuronal excitability (see Riker *et al.*, 1982). However, the clinical literature concerning the effects of long-term exposure to elevated glucocorticoid levels on cognition is certainly consistent with the idea that these steroids can disrupt information processing within certain neural systems. Recent reports on the effects of chronic exposure to stress in the rat suggest that longer exposure to stress can result in electrophysiological (Kerr *et al.*, 1991) and cognitive (Bodnoff *et al.*, 1995) impairments that are not related to neuron loss, but do persist even after the cessation of stress. Bodnoff *et al.* found that the effects of chronic stress are blocked in animals adrenalectomized with low corticosterone replacement, suggesting that it is the increased glucocorticoid levels that alter hippocampal function. Likewise, midaged rats administered high levels of corticosterone for a period of 3 months show greatly reduced hippocampal LTP and impaired spatial memory even 1–2 months following the end of the treatment (Bodnoff *et al.*, 1995; in this study, the LTP deficit was correlated with the degree of cognitive impairment). The effects of stress become more pronounced as the animals age, and in older animals the same chronic stress treatment also produces neuron loss (Kerr *et al.*, 1991; Bodnoff *et al.*, 1995). These findings further underscore the idea that glucocorticoids compromise hippocampal function at several levels, and it appears that the severity of the effects of stress increase with age.

GC receptors and hippocampal aging

Recent data from the laboratories of Slovitar and McEwen have illustrated another dimension to corticosteroid regulation of neuron survival in the hippocampus. In these studies, long-term adrenalectomized animals showed a profound decrease in granule cell density in the dentate gyrus (Slovitar *et al.*, 1989; Gould *et al.* 1992). In this case, the absence of glucocorticoid stimulation in adult animals resulted in neuron loss only in the granule cells of the dentate gyrus. Pyramidal cell density in Ammon's horn (CA1–CA4) was completely unaffected. In contrast, chronic exposure to elevated glucocorticoid levels results in the loss of pyramidal neurons in Ammon's horn, while the dentate is unaffected. Landfield *et al.* (1981) reported that in long-term adrenalectomized animals maintained under conditions of low corticosterone replacement showed no changes in neuron density in the dentate gyrus. The key here is the low level of corticosterone replacement. McEwen's group have recently found that low levels of corticosterone or aldosterone replacement are sufficient to block the effects of adrenalectomy on neuron density in the dentate gyrus (Gould *et al.* 1992). Several in vivo autoradiographic studies have shown that these hormonal treatments will selectively target the mineralocorticoid receptor population in the hippocampus. Thus, the loss of dentate neurons with adrenalectomy (or their maintenance by low levels of corticosterone or aldosterone) appears to be an effect that is mediated by the mineralocorticoid receptor. In contrast, Sapolsky's group (Packan and Sapolsky, 1990) have shown that

the loss of pyramidal neurons in ammon's horn in the presence of elevated glucocorticoid levels is mediated by the glucocorticoid receptor. Thus, the glucocorticoids potentiation of the neurotoxic effects of excitatory amino acids on hippocampal neurons is mimicked by RU28362, a glucocorticoid receptor agonist, and blocked by RU 38486, a glucocorticoid receptor antagonist. Neither of these RU compounds show any binding to mineralocorticoid sites except at very high (i.e., micromolar) concentrations. In essence, low levels of corticoid stimulation are necessary for the maintenance of hippocampal granule neurons, and chronically elevated glucocorticoids are hazzardous for the survival of pyramidal population. The ideal state is obviously one of tight titration around low basal glucocorticoid levels; another important argument for the importance of efficient glucocorticoid negative feedback.

These data suggest that increased activation of glucocorticoid receptors can compromise the survival of hippocampal neurons. The common finding in the aged rat is that of a decrease in glucocorticoid receptor density (with no change in affinity). There are at least two problems in interpreting these findings. First, it is likely that the loss of receptors is secondary to the loss of hippocampal neurons, because corticosteroid-sensitive cells seem to be at risk (see Sapolsky *et al.*, 1985). Second, and perhaps more troubling, is the fact that the measures of receptor density occur some time after the damage has occurred. Existing reports of changes in glucocorticoid receptor density (including our own studies) have been largely performed with 24–26-month-old animals, when hippocampal damaged has already occurred. More pertinent is some indication of changes in glucocorticoid receptors in hippocampal neurons at the time of the insult.

In order to study this question, we have measured changes in glucocorticoid receptor gene expression in animals following lesions to regions that provide major inputs into the hippocampus. Lesions to the medial septum, resulting a major loss of cholinergic input into the hippocampus such as occurs in Alzheimer's disease, produce an increase in hippocampal glucocorticoid and mineralocorticoid receptor mRNA expression (Yau *et al.*, 1992). A comparable phenomenon occurs in striated muscle. Muscle deinnervation severes cholinergic inputs and results in an increase in glucocorticoid receptor density. Interestingly, prolonged exposure to elevated glucocorticoid levels results in muscle wasting. Indeed, the increased tissue sensitivity to circulating glucocorticoids following deinnervation is thought to partly underly the subsequent muscle atrophy.

O'Donnell *et al.* (1993) found that lesions to the entorhinal cortex resulted in a substantial decrease in glucocorticoid receptor mRNA leves in the dentate gyrus, but an increase in the CA1 pyramidal cells. The effect was observed 24 h following the lesion. The lesions result in the loss of a major glutaminergic pathway, and it may be that the loss of these neurons is accompanied by a significant release of glutamate by the dying neurons. It is interesting, therefore, to speculate that the changes in glucocorticoid receptor mRNA occur in response to a substantial pulse of glutamate. Glutamate does result in very rapid changes in glucocorticoid receptor binding both in vivo (Lowy, 1992) and in vitro (O'Donnell, Alonso, and Meaney, unpublished). The nature of this effect is unclear, but it does not seem to involve changes in receptor biosynthesis, because receptor protein levels (as determined using Western blots) are unchanged.

In the course of aging there occurs a loss of hippocampal inputs from the medial septum and the entorhinal cortex. In studies that mimic (albeit in a more acute manner) the loss of these inputs, there occurs an increase in glucocorticoid receptor mRNA in

the CA1 cell field; a region that shows perhaps the greatest age-related loss of neurons in the hippocampus. By contrast, in the dentate gyrus, which is remarkably resistant to neuron loss over the lifespan, there is a decrease in glucocorticoid receptor mRNA following entorhinal cortex lesions. It is, indeed, interesting to presume that these dynamic, acute changes in glucocorticoid receptor gene expression in response to threatening conditions, may, in part at least, determine the sensitivity of cells to circulating glucocorticoids and, thus, regulate neuron survival.

Individual differences in HPA activity and brain aging

Endocrine and psychological responses to stress are notoriously variable across individuals of the same species. This variation often emerges as an important predictor of vulnerability to stress-induced pathology. Over the course of the past years we have been attempting to understanding the neurobiological mechanisms underlying individual differences in HPA responses to stress, and the importance of these differences in HPA function for age-related pathology.

In some of these studies we have used the infant-handling paradigm developed some time ago by Levine, Deneberg, and others (see Meaney *et al.*, 1991b, 1993, for references). In these experiments, rat pups are taken from their nest and handled daily for 15 min over the first 3 weeks of life. The animals are weaned on day 22 of life and handled (H) and nonhandled (NH) rats are then housed under similar conditions until maturity. The HPA profile of the H and NH rats has been reviewed recently, and for the purposes of the present discussion, a short synposis will suffice. As an adult, the H rat shows reduced hypothalamic synthesis of both CRH and AVP (at both the protein and steady-state mRNA levels). These differences are apparent under resting-state conditions. In response to a wide range of stressors, H animals show a more modest increase in plasma ACTH and corticosterone levels than do NH animals.

The mechanism for this difference lies, in part, at the level of the hippocampus and frontal cortex. H rats show increased glucocorticoid (but not nimeralocorticoid) receptor mRNA expression and binding capacity in hippocampus and frontal cortex. In addition, glucocorticoid feedback sensitivity is enhanced in the H rat: Both corticosterone and dexamethasone inhibit ACTH responses to stress more effectively in H rats (i.e., the ID_{50} for both steroids is about 5–10 times lower than in NH rats; Meaney *et al.*, 1989).

The difference in cortical glucocorticoid receptor density is related to the more efficient suppression of poststress HPA activity in the H animals (Meaney *et al.*, 1989b). Chronic administration of corticosterone results in a 30–45% downregulation of hippocampal and frontal cortex glucocorticoid receptor binding sites (Tornello *et al.*, 1982; Sapolsky *et al.*, 1984a,b; Meaney and Aitken, 1985), an effect highly specific to the hippocampus; receptor binding capacity in the hypothalamus and pituitary are unaffected. Using this procedure, we provided evidence for the idea that the changes in hippocampal glucocorticod receptor density is related to the reduced HPA responses to stress in the H animals. Thus, a chronic B treatment regimen that reversed the handling-induced increase in hippocampal and frontal cortex glucocorticoid receptor binding capacity, by downregulating receptor levels to values comparable to those of NH animals, also eliminated the difference in stress-induced levels of corticosterone (Meaney *et al.*, 1989). These data suggest that the difference in negative feedback

efficiency between H and NH is related to the differences in hippocampal and frontal cortex glucocorticoid receptor density. Interestingly, Sapolsky *et al.*, (1990) found that levels of glucocorticoid receptor occupancy in the hippocampus was negatively correlated with hypophysial-portal concentrations of both CRH and AVP. A finding that is analogous to the differences observed between H and NH animals. It appears, then, that the increase in glucocorticoid receptor sites in the hippocampus is a critical feature for the handling effect on HPA function. The increase in receptor density appears to increase the sensitivity of the hippocampus to circulating glucocorticoids, enhancing the efficacy of negative feedback inhibition over HPA activity, and serving to reduce poststress secretion of ACTH and corticosterone in H animals. These differences are apparent as late as 24–26 months of age (Meaney *et al.*, 1988b, 1991a), indicating that the handling effect persists over the entire life of the animal. Thus, we wondered whether H and NH rats might differ in later life on measures of hippocampal function.

Aging rats commonly lose hippocampal corticosteroid receptors, become insensitive to glucocorticoid negative feedback regulation (Sapolsky *et al.*, 1986b), and hypersecrete corticosterone under both basal and poststress conditions. Throughout life, however, H animals have significantly higher hippocampal glucocorticoid receptors than do NH animals (Meaney *et al.*, 1988b, 1991, 1992), although both groups do show a loss of receptors. As expected on the basis of these receptor data, we found that the age-related HPA deficits were far less pronounced in the H rats (Meaney *et al.*, 1988b, 1991). As late as 24 months of age, H animals secrete less corticosterone during restraint stress and terminate corticosterone secretion following stress sooner than did the NH rats.

Moreover, the age-related rise in basal corticosterone levels, often seen in aged rats, occurred in the NH, but not the H animals (Meaney *et al.*, 1988b, 1991, 1992). There is about a twofold increase in basal corticosterone levels in the aged NH animals in the PM phase of the light cycle and about a 40% increase during the AM phase (Meaney *et al.*, 1992). Interestingly, glucocorticoid receptor activation has been associated with negative feedback regulation of PM, but not AM basal ACTH release (see Dallman *et al.*, 1987). In addition, the difference in the PM phase was amplified by the fact that during this time plasma CBG levels in the aged NH animals were significantly lower than in old H animals or young. van Eekelen *et al.* (1991) have also found evidence for decreased plasma CBG levels in aged rats. The decrease in CBG in the old NH animals was associated with a dramatic elevation in free corticosterone to levels (2–4 μg/dl over the entire dark phase of the cycle) that approximated those achieved during stress. This is really an astounding difference. This level of basal free corticosterone suggests that during the PM, the aged NH rats are exposed to the equivalent of 12 h of mild stress in terms of glucocorticoid secretion. Old H animals showed no such decrease in plasma CBG levels and little or no change in basal corticosterone levels. Again, it should be emphasized that these differences in basal corticosterone and CBG between H and NH animals emerge as a function of age: there are no such differences between 6-month-old H and NH animals.

These findings suggest that, over a life span, cumulative exposure to the highly catabolic glucocorticoids is greater in the NH animals. We then examined an important neuropathological consequence of this difference. The reduced cumulative exposure to glucocorticoids in the H rats suggested that hippocampal degeneration would be less pronounced in these animals, and this is exactly what we observed: among NH animals,

but not H animals, there occurred a significant (40%) loss of neurons with age in both the CA1 and CA3 hippocampal cell fields (Meaney *et al.*, 1988b, 1991). Importantly, H and NH rats did not differ in neuron density at 6 months of age. Rather, handling attenuates the loss of hippocampal neurons occurring at later ages.

The difference in hippocampal cell number between the H and NH animals at the later ages was of functional significance. The hippocampus is known to be of considerable importance in learning and memory, and hippocampal injury profoundly disrupts cognition. These findings suggested that the older H rats, with attenuated cell loss in the hippocampus, should show less evidence of age-related cognitive impairments than older NH rats. Behavioral testing was performed using the Morris swim maze (Morris, 1982), a test of spatial memory in which animals are placed into a large (1.6 m), circular pool of opaque water and must use distal, spatial cues to locate a submerged platform onto which they can climb out of the water (rats are proficient, but reluctant swimmers). Spatial memory deficits emerged with age in the NH rats such that the 24-month-old NH animals took significantly longer to locate the platform (i.e., 3–4 times longer) than the 6-month-old animals on all but the first 3 of 18 trials (Meaney *et al.*, 1988b, 1991). In contrast, among the H rats there were no statistically reliable age differences: the 24-month-old H rats performed as well as the 6-month-old animals. There were no differences in the performance of the H and NH animals at 6 months of age. Importantly, in subsequent testing, H and NH rats of all ages performed similarly when the platform was made visible by raising it above the water level, indicating that the H/NH differences were due to spatial, rather than motor skills. These spatial memory deficits in the older NH animals are probably related to the hippocampal damage seen in these animals, as similar deficits are observed after damage to the dorsal hippocampus (e.g., Morris *et al.*, 1982).

Both the present and previous studies have revealed neuroendocrine dysfunction in the aged rat that include glucocorticoid hypersecretion and negative feedback insensitivity, as well as the loss of hippocampal neurons and glucocorticoid receptors. These deficits form a complex and self-perpetuating cascade (see Sapolsky *et al.*, 1986a); a consequence of glucocorticoid hypersecretion is accelerated neuron loss in the aging hippocampus (including corticosterone-concentrating neurons), and a consequence of hippocampal damage is adrenocortical negative feedback insensitivity and glucocorticoid hypersecretion. The interaction of these abnormalities occurs with aging in the rat, and is accelerated by conditions that further elevate glucocorticoid levels, such as stress. Neonatal handling reduces the HPA response to stress. Our data show that this effect persists over the life span. Moreover, one of the critical features of this effect, that of reducing glucocorticoid concentrations under a variety of conditions, appears to prevent the degenerative "glucocorticoid cascade."

The diminished rate of hippocampal neuron loss in the aging H rats probably reflects the lower cumulative lifetime exposure to glucocorticoids. It should be noted that this outcome is the product of two apparently opposing trends. Although the increased concentrations of hippocampal glucocorticoid receptors are related to the enhanced negative-feedback sensitivity and decreased glucocorticoid secretion in the H rats, the same increased receptor concentrations could conceivably sensitize the hippocampus to the endangering effects of glucocorticoids. In this instance, the decreased secretion apparently outweighs the risk of increased target sensitivity, perhaps by ensuring that the prolonged glucocorticoid exposure necessary for the endangering effects does not

occur. It should be noted that under normal resting conditions, only about 10–15% of the hippocampal glucocorticoid receptor population is occupied by hormone (Reul and DeKloet, 1985; Meaney *et al.*, 1988a; Reul *et al.*, 1988). As glucocorticoid levels rise with age, it is the change in hormone levels rather than receptor density that determines the increase in the corticoid signal.

These findings, together with the studies of Landfield, suggest that in a normal population of laboratory rats, individual differences in HPA activity should serve as a predictor of age-related hippocampal pathology. We (Issa *et al.*, 1990) examined this question by screening a large sample of aged (22–28 months) rats using a test of spatial memory. If HPA dysfunction is associated with hippocampal pathology and not merely with advanced age, then we would expect that a sample of aged, cognitively impaired (ACI) and aged, cognitive-unimpaired (ACU), should differ considerably in HPA activity. We used the Morris water maze to screen over 100 aged animals to select aged animals that were either cognitively impaired or unimpaired (> 2 SD or < 0.5 SD from the mean of 6-month-old animals, respectively). According to this criteria, about 30% of the animals were designated as ACI, and a comparable percentage as ACU (underscoring the extreme variation in cognitive decline in aged rats; also see Gage *et al.*, 1984; Gallagher and Pelleymounter, 1988). Interestingly, both groups of aged animals showed a loss of hippocampal neurons; however, the decrease in neuron density was substantially greater in the ACI rats.

The ACI animals showed increased plasma ACTH and corticosterone levels under both basal and poststress conditions, whereas HPA activity in the ACU animals did not differ from 6-month-old controls. As in the old NH animals, the increase in basal ACTH and corticosterone in the ACI rats was observed only in the PM phase of the cycle. Similarly, we found that plasma β-endorphin and cytosolic POMC mRNA levels in the anterior pituitary were elevated in ACI rats during the PM phase (Levin *et al.*, 1992; Sarrieau *et al.*, 1992). Interestingly, while both aged groups showed a loss of hippocampal glucocorticoid receptors (with no change in hypothalamic or pituitary receptor density), the loss was significantly greater in the ACI animals. The ACI rats also showed a significant decrease in hippocampal mineralocorticoid receptor density and decreased mineralocorticoid receptor mRNA. These findings demonstrate the overall loss of hippocampal corticosteroid receptors in the ACI animals. The pattern of data in this study was comparable to that of the previous handling studies, where the old H animals resembled the ACU rats in the Issa *et al.* study. Taken together with the findings of the Landfield studies, these data strongly suggest that increased glucocorticoid levels are selectively associated with the occurrence of hippocampal pathology and impaired cognition in later life.

HPA activity: effects of antidepressant drugs

Perhaps the first insight into the effects of antidepressants on HPA function emerged from the study of endocrine dysfunction in depression. About 50% of depressed patients show evidence for HPA dysregulation: increased cortisol secretion and dampened sensitivity to the negative feedback effects of exogenous glucocorticoid administration, i.e. a reduced dexamethasone-suppression response. Both markers of HPA dysfunction are state dependent and are usually corrected with effective antidepressant treatment (see Chrousos and Gold, 1992). There is now evidence that antidepressant

drugs can, indeed, directly alter HPA function. Prolonged antidepressant drug administration alters CRH gene expression in the PVN and tyrosine hydroxylase gene expression in the locus ceruleus (Brady *et al.*, 1991, 1992). Some of the most intriguing data on this topic have come from the studies of Barden's group with transgenic mice (Pepin *et al.*, 1989, 1991, 1992). These animals have been developed using an antisense for the glucocorticoid receptor, and show decreased levels of glucocorticoid receptor gene expression in brain and pituitary. Not surprisingly, these animals also show increased plasma ACTH and corticosterone levels under basal conditions. Barden and colleagues have shown that antidepressant drugs (i.e., desipramine) increases glucocorticoid receptor gene expression in a variety of brain regions, including the hippocampus and cortex. Similarly, Seckl *et al.* (1992) demonstrated marked increases in both mineralocorticoid and glucocorticoid receptor mRNA expression in the hippocampus, though not parietal cortex, after treatment with antidepressants. Others have also shown an increase in hippocampal mineralocorticoid receptor gene expression with antidepressants. Recently, Reul *et al.* (1993) showed increased hippocampal glucocorticoid and mineralocorticoid receptor binding following chronic treatment with amitryptyline. In each cases cited above, the changes in receptor expression occurred only after chronic treatment.

Barden's lab (Pepin *et al.*, 1989, 1992a, 1992b) found that 10 days of treatment with desipramine resulted in (a) increased glucocorticoid receptor expression in the hypothalamus and hippocampus to virtual normal levels, and (b) greatly attenuated the increase in plasma ACTH and corticosterone normally seen in the transgenic animals. Barden's group have recently shown that the antidepressant drug treatment in the transgemeic animals also increases mineralocorticoid receptor levels in the hippocampus (Barden, personal communication). These findings are consistent with clinical reports that antidepressant drugs correct cortisol hypersecretion and dexamethasone insensitivity in hypercorticoid depressed patients. Thus, antidepressant treatment increase corticosteroid receptor gene expression in brain regions that are known to mediate glucocorticoid negative feedback, and results in decreased HPA hyperactivity. Taken together, these data suggest that antidepressant drug treatment may alter HPA dysfunction, in part at least, by increasing corticosteroid receptor density in brain regions known to mediate the negative feedback effects of circulating glucocorticoids. These studies indicate that antidepressants might at least partially reverse the dysregulation within the HPA axis, and we then became extremely interested in the possibility that these drugs might attenuate the increase pituitary–adrenal activity in the AI rats.

We treated AI, AU, and young rats with either imipramine or vehicle for 5 weeks and examined pituitary–adrenal activity under basal conditions. There were no group differences in plasma ACTH levels in the AM (consistent with our previous reports); nor was there any effect of antidepressant treatment on plasma ACTH levels in the AM in any group. In the PM, AI control animals showed significantly elevated plasma ACTH levels compared with both AU and young animals. Antidepressant drug treatment significantly decreased PM plasma ACTH levels in all animals, and the effect was greatest in the AI: antidepressant treatment virtually eliminated the differences between the AI animals and the AU/young rats. The same pattern was observed for plasma corticosterone levels. The data from the young animals are consistent with those of Reul (1993), who found that amitriptyline decreased plasma ACTH levels even

in young healthy rats, as well as Brady *et al.* (1991, 1992) who found that antidepressant treatment decreased hypothalamic CRH mRNA levels.

Reproductive activity, glucocorticoids, and hippocampal aging

In search of physiologically relevant models for the study of aging, we have come across some older studies examining the relationship between reproductive activity and HPA function. The classic studies here are those with the Pacific salmon, which die shortly after spawning, in a horribly degenerate state promoted by high levels of glucocorticoids (Robertson *et al.*, 1961; Robertson and Wexler, 1966). In humans, as well, there is evidence for Cushing-like states following a life of intense breeding, and the incidence of gestational diabetes atests to the costs of reproduction.

During the latter period of gestation and throughout lactation there are elevated glucocorticoid levels. Thus, differences in the frequency of breeding represents a naturally occurring variation in neuroendocrine history. To study its consequences for aging, we bred female rats 0, 1, 3, or 5 times between 4 and 13 months of age. In each case, breeding was accompanied by the birth of a litter the size of which was in the normal range for the species (i.e., 6–16 pups) and pups were reared to weaning. We found that at 16 months of age, females bred on five occasions (B5) showed increased basal corticosterone levels that were accompanied by decreased plasma CBG levels. At 22 months of age, the pattern was sustained and these females were obese, hypertensive, and quasidiabetic, showing a profile similar to that of the cardiovascular syndrome X. At the same time, however, these females showed no spatial learning impairments, and were, in fact, somewhat better in performance than B0 animals. At the very least, these data indicate that neuropathology does not necessarily occur under conditions of increased HPA activity.

This is not the first report of animals with life histories of increased HPA activity followed by successful brain aging (see Masaro, this volume). Animals fed on restricted diets follow this pattern, although they do not show the degenerate pattern of the B5 females. Whether this finding is exceptional in females is not clear. Our current focus is on the potential role of progesterone as a modulator of glucocorticoid action.

HPA function in human brain aging

In general, increased HPA activity is not associated with aging in humans. However, as in the rat, elevated glucorticoid levels do seem to accompany age-related neuropathology. Studies with multiple samples (as is required in studies of hormones that show circadian rhythms) show that cortisol levels are significantly higher in SDAT patients compared with controls. Davis *et al.* (1986) previously reported significantly higher cortisol levels in SDAT patients, an effect that is most consistent during the late PM–early AM phase of the cycle (i.e., 0200–0900 h). These authors also reported the 0900-h sample discriminated best between SDAT patients and healthy controls. In our own studies, we (Sharma *et al.*, 1989; Nair *et al.*, submitted) found that differences in cortisol levels are more or less consistent across the 24-h cycle and that this is true for both males and females. Moreover, the magnitude of the difference in basal cortisol levels between SDAT patients and controls was substantial (30–52% for males and 57–102% for females), with little change in the pattern of cortisol levels. These findings

suggest that although CNS factors involved in the drive over hypothalamic–pituitary–adrenal activity may be altered in Alzheimer's disease, the signals from the circadian pacemakers that govern the pattern of hormone levels is unchanged.

There may also be changes in plasma CBG levels in SDAT patients. We (Meaney *et al.*, 1989) found a significant (30%) decrease in plasma CBG levels and increased free cortisol concentrations in SDAT patients. Thus, measures of total cortisol levels may actually underestimate the magnitude of the elevated glucocorticoid signal in SDAT patients.

The cause of the increase in basal cortisol levels in SDAT patients is not clear. Resistance to dexamethasone suppression has been reported in a significant number of dementia patients (reviewed in Sapolsky *et al.*,, 1987), which might indicate a dampening of glucocorticoid negative feedback control over HPA function. The report of increased CRF-like immunoreactivity in the paraventricular nucleus of the hypothalamus (PVN) is consistent with this view (Powers *et al.*, 1987), because this region contains the cell bodies of CRF neurons that project into the hypophysial portal system of the anterior pituitary. Bissette *et al.* (1985) found a small and nonsignificant elevation in CRF-like immunoreactivity between Alzheimer's patients and controls. However, this analysis was performed using the whole hypothalamus. Within the hypothalamus, it is only a subpopulation of the CRF-immunoreactive neurons, localized in the medial-parvocellular region, that project to the median eminence and thereby regulate ACTH release (see Swanson *et al.*, 1987). Moreover, this population of CRF-immunoreactive neurons are unique in their sensitivity to glucocorticoids (Swanson *et al.*, 1987). For the study of possible feedback deficits, this is the most relevant population of neurons, and it is not hard to imagine that analysis of whole hypothalamus might have masked differences occurring with this region, such as those detected by Powers *et al.* (1987).

These findings have lead to the hypothesis that increased glucocorticoid levels might promote the loss of hippocampal neurons in later life in humans (see Sapolsky, 1986). It is interesting that hippocampal neuron loss is a hallmark of Alzheimer's disease. Several years ago Sapolsky and Meaney (1988) made a concerted effort to examine the glucocorticoid receptor binding in postmortem human brain tissue. We (Sarrieau *et al.*, 1988) have identified the receptor in biopsy samples of human temporal cortex, but unfortunately, it appears as though the glucocorticoid receptor decays quickly following death in the primate brain. We have recently examined this question using measures of changes in receptor mRNA expression in the postmortem human hippocampus, which shows high expression of both glucocorticoid and mineralocorticoid receptor mRNAs (Seckl *et al.*, 1991). In short, we found little evidence of any change in either glucocorticoid or mineralocorticoid receptor mRNA levels/neuron in Alzheimer brains (Seckl *et al.*, 1993). It should be recalled that there is considerable evidence for increased circulating cortisol levels in Alzheimer patients. These findings suggest that there is no effective, compensatory downregulation of glucocorticoid receptor expression in Alzheimer's disease, perhaps related to the loss of cholinergic input (see Yau *et al.*, 1992). Thus, despite the presence of increased cortisol levels, hippocampal neurons likely remain highly sensitive to glucocorticoids in Alzheimer patients.

In recent studies we have examined the cognitive status of healthy, aged volunteer subjects (average age = 70 years) in relation to basal cortisol levels over the past 4–7 years (Lupien *et al.*, 1994). Prior to neuropsychological testing, subjects were categorized as (a) elevated cortisol levels with a history of increasing levels, (b) moderate–

high levels with a history of increasing levels, or (c) low levels with a tendency for a decline in recent years. The latter group did not differ from young subjects (age = 27 years) in measures of attention of paired-associate learning. In contrast, the two groups with increasing cortisol levels differed on measures of attention and learning of unrelated, but not related, paired associates. This pattern is commonly observed in patients with hippocampal damage. Interestingly, de Leon *et al.*, (1988) and Starkman *et al.* (1992) have found evidence for a relationship between increased cortisol production and hippocampal atrophy among aged subjects. Thus, to date, the existing evidence on the HPA axis and neuropathology in humans is generally consistent with the data from the rodent studies, suggesting a relationship between HPA activity and cognitive status. It is certainly worth mentioning, however, that the studies to date also suggests that prolonged exposure to elevated glucocorticoid levels can damage the primate hippocampus (see Uno *et al.*, 1990; Sapolsky *et al.*, 1990). In addition, studies on cognitive performance of Cushing's patients (Martignoni *et al.*, 1992) have shown that increased glucocorticoid levels are associated with impaired cognitive function in humans.

SUMMARY

Perhaps the critical feature here is the series of events that follow from the loss of glucocorticoid negative feedback sensitivity and the subsequent increase in circulating glucocorticoid levels. It is certainly not our intention to argue that the glucocorticoids are the sole, or even necessarily the principle, regulator of hippocampal pathology in the aged mammal. Rather, the purpose of this review was to illustrate the manner in which peripheral endocrine signals might act to regulate the processes of brain aging. At the same time, in the rodent there is evidence for the idea that increased HPA activity in later life is predictive of hippocampal pathology (measured using both functional and morphological endpoints). In the rat, aged animals with little or no evidence of hippocampal pathology do not differ from young animals in HPA function. In contrast, aged animals demonstrating hippocampal pathology show clearly elevated glucocorticoid secretion (Issa *et al.*, 1990; Sarrieau *et al.*, 1992). These studies underscore the fact that increased HPA activity is not an inevitable consequence of the aging.

Taken together, the results of these studies suggest that increased HPA activity in the elderly might serve as a relevant therapeutic target. Increased glucocorticoid levels impair electrophysiological responses to excitatory input, dampen synaptic regeneration in response to local insult, and promote the loss of hippocampal neurons. Ultimately, it might be reasonable to consider the therapeutic value of treatments that might correct glucocorticoid hypersecretion. Interestingly, one class of drugs that might serve useful in this regard are tricyclic antidepressants. These compounds have been found to increase gluocorticoid receptor mRNA both in vivo (Seckl and Fink, 1992) and in vitro (Pepin *et al.*, 1989), likely through their effects on ascending serotonergic systems (see Mitchell *et al.*, 1990; Seckl *et al.*, 1990, 1991). An increase in glucocorticoid receptor sites should increase negative feedback sensitivity and reduce HPA activity.

The role of glucocorticoids in potentiating excitotoxic damage to hippocampal neurons has been well established in the studies of Sapolsky and others. What is less clear is the nature of the neurotoxic signal(s) when damage occurs with aging and the immediate response of cortical neurons to threatening conditions, where there appears to

be considerably regional variation. However, in response to the loss of either septal or entorhinal inputs that can occur with aging, there is an increase in glucocorticoid receptor mRNA expression in the CA1 cell field. An increase in the biosynthesis of glucocorticoid receptors under these conditions coupled with increased HPA activity would seem to pose a risk for the function and the survival of these neurons.

REFERENCES

AITKEN, D.H. and MEANEY, M.J. Temporally graded, age-related impairments in spatial memory in the rat. *Neurobiol. Aging* **10,** 273–277, 1990.

ANTONI, F.A. Hypothalamic control of ACTH secretion: Advances since the discovery of 41-residue corticotropin-releasing factor. *Endocr. Rev.* **7,** 351–370, 1986.

ARMANINI, M., HUTCHINS, C., STEIN, B., and SAPOLSKY, R.M. Glucocorticoid endangerment of hippocampal neurons is NMDA-receptor dependent. *Brain Res.* **20532,** 7–13, 1990.

BAXTER J. D. and TYRRELL J. B. The adrenal cortex. In: *Endocrinology and Metabolism*, Felig, P., Baxter, J. D., Broadus, A. E., Frohman, L. A. (Editors), pp. 385–511, McGraw-Hill, New York, 1987.

BENNET, M.C., DIAMOND, D.M., FLESHNER, M., and ROSE, G.M. Serum corticosterone level predicts the magnitude of hippocampal primed burst potentiation and depression in urethane-anesthetized rats. *Psychobiology* **19,** 301–307, 1991.

BENSTON, J. REZA, M.R., WINTER, J., and WILSON, G. Steroids and apparent cerebral atrophy on computed tomography scans. *J. Comput. Asst. Tomogr.* **2,** 16–23, 1978.

BISSETTE, G., REYNOLDS, G.P., KILTS, C.D., and NEMEROFF, C. Corticotropin-releasing factor-like immunoreactivity in senile dementia of the Alzheimer type. *JAMA* **254,** 3067–3069, 1985.

BODNOFF, S.R., HUMPHREYS, A.G., LEHMAN, J.C., DIAMOND, D.M., ROSE, G.M., and MEANEY, M.J. Enduring effects of chronic corticosterone treatment on spatial learning, synaptic plasticity and hippocampal neuropathology in young and mid-aged rats. *J. Neurosci.* **15,** 61–69, 1995.

BRADBURY, M.J., AKANA, S.F., and DALLMAN, M.F. Roles of type I and type II corticosteroid receptors in regulation of basal activity in the hypothalamo–pituitary–adrenal axis during the dirunal trough and the peak: Evidence for a nonadditive effect of combinded receptor occupation. *Endocrinology,* **134,** 1286–1297, 1994.

BRADY, L.S., WHITFIELD, H.W., FOX, R., GOLD, P.W., and HERKENHAM, M. Antidepressant drugs down-regulate corticotrophin releasing factor and tyrosine hydroxylase messenger RNAs in rat brain. *J. Clin. Invest.* **87,** 831–837, 1991.

BRADY, L.S., GOLD, P.W., HERKENHAM, M., LYNN, A.B., and WHITFIELD, H.W. The antidepressants fluoxetine, idazoxan and phenelzine alter corticotropin-relasing hormone and tyrosine hydroxylase mRNA levels in rat brain: Therapeutic implications. *Brain Res.* **572,** 117–125, 1992.

BRINDLEY, D.N. and ROLLAND, Y. Possible connections between stress, diabetes, obesity, hypertension and altered lipoprotein metabolism that may result in atherosclerosis. *Clin. Sci.* **77,** 453–461, 1989.

CARNES, M., GOODMAN, B.M., LENT, S.J, and VO, H. High intensity venous sampling reveals subtle alterations in plasma adrenocorticotropin patterns in old rats. *Endocrinology,* **133,** 608–616, 1993.

CHROUSOS, G.P. and GOLD, P.W. The concept of stress and stress system disorders. *JAMA* **267,** 1244–1252, 1992.

CIZZA, G., CALOGERO, A.E., BRADY, L.S., BAGDY, G., BERGAMINI, E., BLACKMAN, M.R., CHROUSOS, G.P., and GOLD, P.W. Male Fisher 344/N rats show a progressive central impairment of the hypothalamic–pituitary–adrenal axis with advancing age. *Endocrinology* **134,** 1611–1620, 1994.

COLEMAN, P.D. and FLOOD, D.G. Neuron numbers and dendritic extent in nornal aging and Alzheimer's Disease. *Neurobiol. Aging* **8,** 521–545, 1987.

DALLMAN, M.F., AKANA, S., CASCIO, C.S., DARLINGTON, D.N., JACOBSON, L., and LEVIN, N. Regulation of ACTH secretion: Variations on a theme of B. *Rec. Prog. Horm. Res.* **43,** 113–173, 1987.

DALLMAN, M.F., AKANA, S.F., SCRIBNER, K.A., BRADBURY, M.J., WALKER, C.-D., STRACK, A.M., and CASCIO, C.S. Stress, feedback and facilitation in the hypothalamo–pituitary–adrenal axis. *J. Neuroendocrinol.* **4,** 517–526, 1993.

DAVIS, K.L., DAVIS, B. M., GREENWALD, B.S., MOHS, R.C., MATHE, A.A., JOHNS, C.A., and HORVATH, T.B. Cortisol and Alzheimer's disease, I: Basal studies. *Am. J. Psychiatry* **143,** 300–305, 1986.

DE LEON, M.J., MC RAE, T., TSAI, J.R., GEORGE, A.E., MARCUS, D.L., FREEDMAN, M., WOLF, A.P., and MC EWEN, B.S. Abnormal cortisol response in Alzheimer's disease linked to hippocampal atrophy. *Lancet* **ii,** 391–392, 1988.

DE KLOET, E.R Brain corticosteroid receptor balance and homeostatic control. *Front. Neuroendocrinol.* **12,** 95–164, 1991.

DE KOSKY, S.T., SCHEFF, S.W., and COTMAN, C.W. Elevated corticosterone levels: A possible cause of reduced axon sprouting in aged animals. *Neuroendocrinology* **38,** 33–38, 1984.

DIAMOND, D.M., BENNET, M.C., STEVENS, K.E., WILSON, R.L., and ROSE, G.M. Exposure to a novel environment interferes with the induction of hippocampal primed burst potentiation. *Psychobiology* **18,** 273–281, 1990.

DIAMOND, D.M., BENNET, M.C., FLESHNER, M., and ROSE, G.M. Inverted-U relationship between the level of peripheral corticosterone and the magnitude of hippocampal primed burst potentiation. *Hippocampus* **2,** 421–430, 1992.

DILMAN, V. *The Law of Deviation of Homeostasis and Diseases of Aging,* John Wiley, Boston, 1981.

FOY, M.R., STANTON, M.E., LEVINE, S., AND THOMPSON, R.F. Behavioral stress impairs long-term potentiation in rodent hippocampus. *Behav. Neural Biol.* **48,** 138–149, 1987.

FUNDER, J.W. and SHEPPARD, K. Adrenocortical steroids and the brain. *Annu. Rev. Physiol.* **49,** 397–412, 1987.

GAGE, F.H., KELLY, P.A.T., and BJORKLUND, A. Regional changes in brain glucose metabolisn reflect cognitive impairments in age rats. *J. Neurosci.* **4,** 2856–2865, 1984.

GALLAGER, M. and PELLEYMOUNTER, M. Spatial learning deficits in old rats: A model for memory decline in the aged. *Neurobiol. Aging* **9,** 549–556, 1988.

GOULD, E., WOOLLEY, C., and MC EWEN, B.S. Short-term glucocorticoid manipulations affect neuronal morphology and survival in the adult dentate gyrus. *Neuroscience* **37,** 367–375, 1992.

HAUGER, R.L., THRIVIKRAMAN, K.V., and PLOTSKY, P.M. Age-related alterations of hypothalamic–pituitary– adrenal axis function in male Fisher 344 rats. *Endocrinology* **134,** 1528–1536, 1994.

HEINZ, E.R., MARTINEZ, J., and HAENGGELI, A. Reversibility of cerebral atrophy in anorexia nervosa and Cushing's Syndrome. *J. Comput. Assist. Tomogr.* **1,** 415–418, 1977.

HESS, G.D. and REIGLE, G.D. Adrenocortical responsiveness to stress and ACTH in aging rats. *J. Gerontol.* **25,** 253–270, 1970.

HORNER, H., PACKAN, D., and SAPOLSKY, R.M. Glucocorticoids inhibit glucose transport in cultured hippocampal neurons and glia. *Neuroendocrinology* **52,** 57–64, 1990.

ISSA, A., ROWE, W. GAUTHIER, S., and MEANEY, M.J. Hypothalamic–pituitary–adrenal activity in aged cognitively impaired and cognitively unimpaired aged rats. *J. Neurosci.* **10,** 3247–3254, 1990.

JACOBSON, L. and SAPOLSKY, R.M. The role of the hippocampus in feedback regulation of the hypothalamic–pituitary–adrenal axis. *Endocr. Rev.* **12,** 118–134, 1991.

JOELS, M. and DE KLOET, E.R. Effect of glucocorticoids and norepinepherine on excitability in the hippocampus. *Science* **245,** 1502–1504, 1989.

JOELS, M. and DE KLOET, E.R. Mineralocorticoid receptor-mediated changes in membrane properties of rat CA1 pyramidal neurons in vitro. *Proc. Natl. Acad. Sci. USA* **87,** 4495–4498, 1990.

JONES, M.T., GILLHAM, B., GREENSTEIN, B.D., BECKFORD, U., and HOLMES, M.C. Feedback actions of adrenal steroid hormones. In: *Current Topics in Neuroendocrinology,* vol. 2, Ganten, D. and Pfaff, D. (Editors), pp. 45–68, Springer Verlag, New York, 1982.

KADEKARO, M., MASANORI, I., and GROSS, P. Local cerebral glucose utilization is increased in acutely adrenalectomized rats. *Neuroendocrinology* **47,** 329–337, 1986.

KELLER-WOOD, M. and DALLMAN, M.F. Corticosteroid inhibition of ACTH secretion. *Endocr. Rev.* **5,** 1–24, 1984.

KERR, D.S., CAMPBELL, L.W., HAO, S.-Y., and LANDFIELD, P.W. Corticosteroid modulation of hippocampal potentials: Increased effect with aging. *Science* **245,** 1505–1509, 1989.

KERR, D.S., CAMPBELL, L.W., APPLEGATE, M.D., BRODISH, A., and LANDFIELD, P.W. Chronic stress-induced acceleration of electrophysiologic and morphometric biomarkers of hippocampal aging. *J. Neurosci.* **11,** 1316–1324, 1991.

LANDFIELD, P., BASKIN, R.K., and PITLER, T.A. Brain-aging correlates: Retardation by hormonal-pharmacological treatments. *Science* **214,** 581–583, 1981.

LANDFIELD, P.W. and PITLER, T.A. Prolonged Ca-dependent afterhyperpolarizations in hippocampal neurons of the aged rat. *Science* **226,** 1089–1092, 1984.

LEVIN, N., BENGANI, N., ROWE, W., MEANEY, M.J., and ROBERTS, J.L. Pituitary POMC mRNA levels in aged-impaired and aged-unimpaired rats. *Soc. Neurosci. Abstr.* **18,** 668, 1992.

LOWY, M.T. Kainic acid induced decrease in hippocampal corticosteroid receptors. *J. Neurochem.* **58,** 1561–1568, 1992.

LUPIEN, S., LECOURS, A.R., SCHWARTZ, G., SHARMA, S., NAIR, N.P.V., and MEANEY, M.J. Neuropsychological assessment of elderly subjects in relation to basal cortisol levels. *J. Neurosci.* **14,** 2893–2903, 1994.

MARTGNONI, E., COSTA, A., SINFORIANI, E., LUIZZI, A., CHIODINI, P., MAURI, M., BONO, G., and NAPPI, G. The brain as a target for adrenocortical steroids: Cognitive implications. *Psychoneuroendocrinology* **17,** 343–354, 1992.

MC EWEN, B.S., DE KLOET, E.R., and ROSTENE, W.H. Adrenal steroid receptors and actions in the nervous system. *Physiol. Rev.* **66,** 1121–1150, 1986.

MEANEY, M.J. and AITKEN, D.H. [^{3}H]Dexamethasone binding in rat frontal cortex. *Brain Res.* **328,** 176–180, 1985.

MEANEY, M.J., VIAU, V., BHATNAGAR, S., and AITKEN, D.H. Occupancy and translocation of hippocampal glucocorticoid receptors during and following stress. *Brain Res.* **445,** 198–203, 1988a.

MEANEY, M.J., AITKEN, D.H., BHATNAGAR, S., VAN BERKEL, CH., and SAPOLSKY, R.M. Postnatal handling attenuates neuroendocrine, anatomical, and cognitive impairments related to the aged hippocampus. *Science* **238,** 766–768, 1988b.

MEANEY, M.J., AITKEN, D.H., SHARMA, S., VIAU, V., and SARRIEAU, A. Postnatal handling increases hippocampal type II, glucocorticoid receptors and enhances adrencocortical negative-feedback efficacy in the rat. *Neuroendocrinology* **50,** 597–604, 1989.

MEANEY, M.J., SHARMA, M., SHARMA, S., THAKUR, M., QUIRION, R., SAPOLSKY, R.M., and NAIR, N.P.V. Hypothalamic–pituitary–adrenal function in Alzheimer patients and aged controls. *Soc. Neurosci. Abstr.* **15,** 1043, 1989.

MEANEY, M.J., AITKEN, D.H., and SAPOLSKY, R.M. Environmental regulation of the adrenocortical stress response in female rats and its implications for individual differences in aging. *Neurobiol. Aging* **12,** 31–38, 1991a.

MEANEY, M.J., MITCHELL, J.B., BHATNAGAR, S., INY, L.J., BODNOFF, S.R., VIAU, V., and SARRIEAU, A. The effects of neonatal handling on the development of the adrenocortical stress response: Implications for neuropathology and cognitive deficits in later life. *Psychoneuroendocrinology* **16,** 87–105, 1991b.

MEANEY, M. J., AITKEN, D. H., SHARMA, S., and VIAU, V. Basal ACTH, corticosterone, and corticosterone-binding globulin levels over the diurnal cycle, and hippocampal type I and type II corticosteroid receptors in young and old, handled and nonhandled rats. *Neuroendocrinology* **55,** 204–213, 1992.

MEANEY, M.J., BHATNAGAR, S., MC CORMICK, C.M., SHANKS, N., SMYTHE, J.W., and VIAU, V. Individual differences in hypothalamic–pituitary–adrenal stress response and the hypothalamic CRF system. *Ann. NY Acad. Sci. 697,* 70–86, 1993.

MITCHELL, J. B., ROWE, W., BOKSA, P., and MEANEY, M.J. Serotonin regulates type II corticosteroid receptor binding in hippocampal cell cultures. *J. Neurosci.* **10,** 1524–1533, 1990.

MORRIS, R.G.M., GARRARD, P., RAWLINS, J.N.P., and O'KEEFE, J. Place navigation is impaired in rats with hippocampal lesions. *Nature* **297,** 681–683, 1982.

MUNCK, A., GUYRE, P.M., and HOLBROOK, N.J. Physiological functions of glucocorticoids in stress and their relations to pharmacological actions. *Endocr. Rev.* **5,** 25–44, 1984.

NAIR, N.P.V., SHARMA, M., THAKUR, M., SCHWARTZ, G., GHADIRIAN A., QUIRION, R., and MEANEY, M.J. Circadian rhythms of cortisol and melatonin in Senile Dementia of Alzheimer's Type (SDAT). (submitted).

O'DONNELL, D., BACCHIET, A., SECKL, J.R., MEANEY, M.J., and POIRIER, J. Entorhinal cortex lesions alter hippocampal glucocorticoid but not mineralocorticoid receptor mRNA levels. *J. Neurochem.* **61,** 356–359, 1993.

OITZL, M.S. and DE KLOET, E.R. Selective corticosteroid antagonists modulate specific aspects of spatial orientation learning. *Behav. Neurosci.* **106,** 62–71, 1992.

OKUNO, T., ITO, M., KONISHI, Y., YOSHIOKA, Y., and NAKANO, Y. Cerebral atrophy following ACTH therapy. *J. Comput. Assist. Tomogr.* **4,** 20–23, 1980.

PACKAN, D. and SAPOLSKY, R.M. Glucocorticoid endangerment of the hippocampus: Tissue, steroid, and receptor specificity. *Neuroendocrinology* **51,** 613–618, 1990.

PEIFFER, A., BARDEN, N., and MEANEY, M.J. Age-related changes in glucocorticoid receptor binding and mRNA levels in the rat brain and pituitary. *Neurobiol. Aging* **12,** 475–479, 1991.

PEPIN, M.-C., BEAULIEU, S., and BARDEN, N. Antidepressants regulate glucocorticoid receptor messanger RNA concentrations in primary neuronal cultures. *Mol. Brain Res.* **6,** 77–83, 1989.

PEPIN, M.C., POTHIER, F., and BARDEN, N. Impaired type II glucocorticoid receptor function in mice bearing antisense RNA transgene. *Nature* **355,** 725–728, 1992a.

PEPIN, M.C., GOVIDAN, M., and BARDEN, N. Increased glucocorticoid receptor gene promoter activity after antidepressant treatment. *J. Pharmacol. Exp. Ther.* **41,** 1016–1024, 1992b.

PFAFF, D.W., SIVA, M.T., and WEISS, J.M. Telemetered recording of hormone effects on hippocampal neurons. *Science* **172,** 394–395, 1971.

PLOTSKY, P.M. Regulation of hypophysiotropic factors mediating ACTH secretion. *Ann. NY Acad. Sci.* **512,** 205–217, 1987.

PLOTSKY, P.M. and VALE, W.W. Hemorrhage-induced secretion of corticotropin-releasing factor-like immunoreactivity into the rat hypophysial portal circulation and its inhibition by glucocorticoids. *Endocrinology* **114,** 164–169, 1984.

POWERS, R.E., WALKER, L.C., DESOUZA, E.B., VALE, W.W., STRUBLE, R.G., WHITEHOUSE, P.J., and PRICE, D.L. CRF-like immunoreactivity in the CNS of Alzheimer's Disease. *Synapse* **1,** 405–414, 1987.

RAPAPORT, P.A., ALLAIRE, Y., BOURLIERE, F., and GIRARD, F. Reactivite au "stress" et capacite d'adaptation a une situation inhabituelle chez le rat jeune, adulte et age. *Gerontologia* **10,** 20–30, 1964.

REUL, J.M.H.M. and DE KLOET, E.R. Two receptor systems for corticosterone in rat brain: Microdistribution and differential occupation. *Endocrinology* **117,** 2505–2511, 1985.

REUL, J.M.H.M., TONNAER, J., and DE KLOET, E.R. Neurotrophic ACTH analgue promotes plasticity of type I corticosteroid receptors in brain of senescent male rats. *Neurobiol. Aging* **9,** 253–257, 1988.

REUL, J.M.H.M., STEC, I., SODER, M., and HOLSBOER, F. Chronic treatment of rats with the antidepressant amitripyline attenuates the activity of the hypthalamic–pituitary–adrenocortical system. *Endocrinology* **133,** 312–319, 1993.

RIKER, W.F., BAKER, T., and SASTRE, A. Electrophysiologic and clincial aspects of glucocorticoids on certain neural systems. In: *Current Topics in Neuroendocrinology,* vol. 2, Ganten, D. and Pfaff, D. (Editors), pp. 69–105, Springer-Verlag, New York, 1982.

RITGER, H., VELDHUIS, H., and DE KLOET , E.R. Spatial orietation and hippocampal corticosterone receptor systems of old rats: Effects of ACTH4–9 analogue ORG2766. *Brain Res.* **309,** 393–399, 1984.

RIVIER, C. and PLOTSKY, P. M. Mediation by corticotropin-releasing factor of adenohypophysial hormone secretion. *Annu. Rev. Physiol.* **48,** 475–489, 1986.

ROBERTSON, O.H., KRUPP, M.A., THOMAS, S.P., FAVOUR, C.B., HANE, S., and WEXLER, B.C. Hyperadrenocorticism in spawning migratory and nonmigratory rainbow trout; Comparison with Pacific Salmon. *Gen. Comp. Endocrinol.* **1,** 473–484, 1961.

ROBERTSON, O.H. and WEXLER, B.C. Histological changes in the organs and tissues of migrating and spawning Pacific Salmon. *Endocrinology* **66,** 222–239, 1966.

ROWE, J.W. and KAHN, R.L. Human aging: Usual and successful. *Science* **237,** 143–151, 1987.

RUBINOW, D.R., POST, R.M., SAVARD, R., and GOLD, P.W. Cortisol hypersecretion and cognitive impairment in depression. *Arch. Gen. Psychiatry* **41,** 279–283, 1984.

SAPOLSKY, R.M. A mechanism for increased glucocorticoid toxicity in the hippocampus: Increased neuronal vulnerability to metabolic insults. *J. Neurosci.* **5,** 1227–1232, 1985.

SAPOLSKY, R.M. Glucocorticoid toxicity in the hippocampus: Reversal with supplementation with brain fuels. *J. Neurosci.* **6,** 2240–2245, 1986.

SAPOLSKY, R.M. Glucocorticoids, hippocampal damage and the glutaminergic synapse. *Prog. Brain Res.* **86,** 13–23, 1990.

SAPLOLSKY, R.M. Do glucocorticoid levels rise with age in the rat? *Neurobiol. Aging* **13,** 171–176, 1991.

SAPOLSKY, R.M. and MEANEY, M.J. Postmortem decay in glucocorticoid binding in human and primate brain. *Brain Res.* **448,** 182–185, 1988.

SAPOLSKY, R.M., KREY, L.C., and MC EWEN, B.S. The adrenocortical response in the aged rat: Impairment of recovery from stress. *J. Exp. Gerontol.* **18,** 55–63, 1983a.

SAPOLSKY, R.M., KREY, L.C., and MC EWEN, B.S. Corticosterone receptors decline in a site-specific manner in the aged rat. *Brain Res.* **289,** 235–240, 1983b.

SAPOLSKY, R.M., KREY, L.C., and MC EWEN, B.S. Glucocorticoid-sensitive hippocampal neurons are

involved in terminating the adrenocortical stress response. *Proc. Natl. Acad. Sci. USA* **81,** 6174–6177, 1984a.

SAPOLSKY, R.M., KREY, L.C., and MC EWEN, B.S. Stress downregulated corticosterone receptors in a site-specific manner. *Endocrinology* **114,** 287–292, 1984b.

SAPOLSKY, R.M., KREY, L.C., and MC EWEN, B.S. Prolonged glucocorticoid exposure reduced hippocampal neuron number: Implications for aging. *J. Neurosci.* **5,** 1221–1226, 1985.

SAPOLSKY, R.M., KREY, L.C., and MC EWEN, B.S. The neuroendocrinology of stress and aging: The glucocorticoid cascade hypothesis. *Endocr. Rev.* **7,** 284–301, 1986a.

SAPOLSKY, R.M., KREY, L.C., and MC EWEN, B.S. The adrenocortical axis in the aged rat: Impaired sensitivty to both fast and delayed feedback inhibition. *Neurobiol. Aging* **7,** 331–336, 1986b.

SAPOLSKY, R.M., ARMANINI, M., PACKAN, D., and TOMBAUGH, G. Stress and glucocorticoids in aging. *Endocr. Metab. Clin.* **16,** 965–980, 1987.

SAPOLSKY, R.M., ARMANINI, M.P., PACKAN, D.R., SUTTON, S.W., and PLOTSKY, P.M. Glucocorticoid feedback inhibition of adrenocorticotropic hormone secretagogue release: Relationship to corticosteroid receptor occupancy in various limbic sites. *Neuroendocrinology* **51,** 328–336, 1990.

SARRIEAU, A., DUSSAILLANT, M., SAPOLSKY, R.M., AITKEN, D.H., OLIVIER, O., LAL, S., ROSTENE, W.H., QUIRION, R., and MEANEY, M.J. Glucocorticoid binding sites in human temporal cortex. *Brain Res.* **442,** 159–163, 1988.

SARRIEAU, A., ROWE, W., O'DONNELL, D., LEVIN, N., SECKL, J.R., and MEANEY, M.J. Increased hypothalamic ACTH secretagogue synthesis in aged impaired vs. aged unimpaired rats. *Soc. Neurosci. Abstr.* **18,** 669, 1992.

SECKL, J.R., DICKSON, K.L., and FINK, G. Central 5,7-dihydroxytryptamine lesions decrease hippocampal glucocorticoid and mineralocorticoid receptor messanger ribonucelic acid expression. *J. Neuroendocrinol.* **2,** 911–916, 1990.

SECKL, J.R., YAU, J.L.W., and FINK, G. Distribution of glucocorticoid and mineralocorticoid receptor messanger RNA expression in human hippocampus. *Brain Res.* **561,** 332–337, 1991.

SECKL, J.R. and FINK, G. Antidepressants increase glucocorticoid and mineralocorticoid receptor mRNA expression in rat hippocampus in vivo. *Neuroendocrinology* **55,** 621–626, 1992.

SECKL, J.R., FRENCH, K.L., O'DONNELL, D., MEANEY, M.J., NAIR, N.P.V., YATES, C.M., and FINK, G. Glucocorticoid receptor gene expression is unaltered in hippocampal neurons in Alzheimer's disease. *Mol. Brain Res.* **18,** 239–245, 1993.

SEISJO, B. Cell damage in the brain: A speculative synthesis. *J. Cereb. Blood Flow Metab.* **1,** 155–175, 1981.

SENCAR-CUPOVIC, I. and MILKOVIC, S. The development of sex differences in adrenal morphology and responsiveness to stress of rats from birth to end of life. *Mech. Ageing Dev.* **5,** 1–9, 1976.

SHARMA, M., PALACIOS-BOIS, J., SCHWARTZ, G., ISKANDAR, H., THAKUR, M., QUIRION, R., and NAIR, N.P.V. Circadian rhythms of malatonin and cortisol in aging. *Biol. Psychiatry* **25,** 305–319, 1989.

SHEPPARD, K.E. and FUNDER, J.W. Equivalent affinity of aldosterone and corticosterone for type I receptors in kidney and hippocampus: Direct binding studies. *J. Steroid Biochem. Mol. Biol.* **28,** 737–742, 1987.

SLOVITAR, R., VALIQUETTE, G., ABRAMS, G., RONK., E., SOLLAS, A., PAUL., L., and NEUBORT, S. Selective loss of hippocampal granule cells in the mature rat brain after adrenalectomy. *Science* **243,** 535–538, 1989.

SONNTAG, W.E., GOLIESZEK, A.G., BRODISH, A., and ELDRIDGE, J.C. Diminished diurnal secretion of adrenocorticotropin (ACTH), but not corticosterone, in old male rats: Possible relation to increased adrenal sensitivity to ACTH in vivo. *Endocrinology* **120,** 2308–2315, 1987.

STARKMAN, M.N., SCHTEINGART, D.E., and SCHORK, M. Depressed mood and other psychiatric manifestations of Cushing's Syndrome: Relationship to hormones. *Psychosom. Med.* **43,** 3–18, 1981.

STARKMAN, M.N. and SCHTEINGART, D.E. Neuropsychiatric manifestations of patients with Cushings's syndrome. *Arch. Intern. Med.* **141,** 215–218, 1981.

STARKMAN, M.N., GEBARSKI, S.S., BERENT, S., and SCHEINGART, D.E. Hippocampal formation volume, memory dysfunction, and cortisol levels. *Biol. Psychiatry* **32,** 756–765, 1992.

SWANSON, L.W., SAWCHENKO, P.E., LIND, R.W., and RHO, J.H. The CRH motoneuron: Differential peptide regulation in neurons with possible synaptic, paracrine, and endocrine outputs. *Ann. NY Acad. Sci.* **512,** 12–23, 1987.

TANG, F. and PHILIPS, J.G. Some age-related changes in pituitary-adrenal function in the male laboratory rat. *J. Gerontol.* **33,** 377–382, 1978.

TORNELLO, S., ORTI, E., DENICOLA, A.F., RAINBOW, T.C., and MC EWEN, B.S. Regulation of glucocorticoid receptors in brain by corticosterone treatment of adrenalectomized rats. *Neuroendocrinology* **35,** 411–417, 1982.

UNO, H., TARARA, R., ELSE, J., SULEMAN, M., and SAPOLSKY, R.M. Hippocampal damage associated with prolonged and fatal stress in primates. *J. Neurosci.* **9,** 1705–1711, 1989.

UNO, H., LOHMILLER, L., THIEME, C., KEMNITZ, J., ENGLE, M., ROECKER, E., and FARRELL, P. Brain damage induced by prenatal exposure to dexamethasone in fetal rhesus macaques: Hippocampus. *Dev. Brain Res.* **53,** 157–167, 1990.

VAN EEKELEN, J.A.M., ROTS, N.Y., SUTANTO, W., and DE KLOET, E.R. The effect of aging on stress responsiveness and central corticosteroid receptors in the Brown Norway rat. *Neurobiol. Aging* **13,** 159–170, 1991.

VIRGIN, C.E., HA, T.P.-T., PACKAN, D., TOMBAUGH, G., YANG, S., HORNER, H.C., and SAPOLSKY, R.M. Glucocorticoids inhibit glucose transport and glutamate uptake in hippocampal astrocytes: Implications for glucocorticoid neurotoxicity. *J. Neurochem.* **57,** 1422–1428, 1991.

WHELAN, T.B., SCHTEINGART, D.E., STARKMAN, M.N., and SMITH, A. Neuropsychological deficts in Cushing's Syndrome. *J. Nerv. Mental Dis.* **168,** 753–757, 1980.

YAU, J.L.W., DOW, R.C., FINK, G., and SECKL, J.R. Medial septal cholinergic lesions increase hippocampal mineralocorticoid and glucocorticoid receptor messanger RNA expression. *Brain Res.* **577,** 155–160, 1992.

YAU, L.W., OLSSON, T., MORRIS, R.G.M., MEANEY, M.J., and SECKL, J.R. Glucocorticoids corticosteroid receptor gene expression and antidepressant treatment; Relationship with spatial learning in young and aged rats. *Neuroscience* (in press).