Hypothalamic-Pituitary-Adrenal Activity in Aged, Cognitively Impaired and Cognitively Unimpaired Rats

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There is a tendency for increased hypothalamic-pituitary-adrenal (HPA) activity with age in the rat, and the resulting elevations in circulating glucocorticoid levels have been implicated in the occurrence of hippocampal pathology and memory deficits. In the experiments reported here, we examined whether HPA dysfunction is selectively associated with cognitive impairments in a population of aged rats. Fifty-eight 23–27-month-old male Long-Evans rats were screened for spatial memory impairments using the Morris swim maze, and 2 groups of aged animals were selected: aged, cognitively impaired (AI) animals whose performance was significantly different (>2 SD) from that of 6-month-old controls and aged, cognitively unimpaired (AU) animals whose performance was comparable to that of the young controls (a difference of <0.5 SD). Twenty-eight percent of the animals tested were designated as AI and 20% as AU. Histological analysis of a subset of these animals showed that, while both AU and AI animals showed neuron loss in the pyramidal cell fields of the hippocampus, the loss was significantly greater in the AI animals. The AI animals showed clear evidence of increased HPA activity. Thus, basal ACTH and corticosterone levels were significantly higher in the AI animals compared with both AU animals and young controls, especially during the dark phase of the cycle. The AI, AU, and young animals exhibited comparable corticosterone levels during a 20-min immobilization stress; however, following the termination of the stressor, corticosterone levels in AI animals were significantly elevated compared with both AU animals and controls. The AU animals did not differ from the young controls on any measure of HPA function. These findings suggested impaired glucocorticoid negative feedback in the AI animals. Both AU and AI animals showed reduced hippocampal type I and type II corticosteroid-receptor binding; however, the reduction was significantly greater in the AI animals. There were no differences in receptor binding in the hypothalamus or pituitary, and these findings are consistent with the idea that age-related HPA dysfunction is associated with the loss of hippocampal corticosteroid receptors. These data suggest that HPA dysfunction among aged animals is selectively associated with spatial memory impairments and increased hippocampal neuron loss and does not emerge merely as a function of age.

Basal levels of hypothalamic-pituitary-adrenal (HPA) activity tend to increase with age in the rat, resulting in elevated plasma levels of ACTH and corticosterone (Sencar-Cupovic and Milkovic, 1976; Tang and Philips, 1978; Brett et al., 1983, 1986; Sapolsky et al., 1983a; DeKosky et al., 1984; Meaney et al., 1988a, 1990a; but see also Sonntag et al., 1987). This increase in HPA activity appears to occur as a function of dampened glucocorticoid negative-feedback inhibition over HPA activity. Thus, aged rats showed reduced HPA suppression following exogenous treatment with either corticosterone (Sapolsky et al., 1986a) or dexamethasone (Dilman, 1981). The decreased glucocorticoid inhibition of HPA activity is, in turn, likely associated with a loss of corticosteroid receptors in certain brain regions that mediate the negative-feedback effects of circulating glucocorticoids. Specifically, there is a decrease of both type I (mineralocorticoid-like) and type II (glucocorticoid) corticosteroid receptors in the hippocampus of the aged rat (Sapolsky et al., 1983b; Ritger et al., 1984; Meaney et al., 1988a, 1990a,b; Reul et al., 1988).

The increased exposure to the highly catabolic adrenal glucocorticoids appears to be associated with the loss of hippocampal neurons and the emergence of cognitive deficits in the aged rat. The hippocampus contains the greatest density of corticosteroid receptors in the brain (McEwen et al., 1986), and it seems that this high sensitivity to adrenal steroids in the presence of the elevated corticosterone titers often seen in aged rats promotes hippocampal neuron loss, especially the loss of pyramidal cells. [Note that this effect may depend on the age of the animal, as peripubertal rats show granule cell loss in the absence of glucocorticoids; see Slovitar et al. (1989).] Among a population of aged rats, the magnitude of hippocampal neuron loss and memory deficits is positively correlated with the increase in adrenal activity (Landfield et al., 1978). Moreover, animals adrenalectomized at midlife (with low-level corticosterone replacement) show reduced neuron loss in the hippocampus and improved cognitive functioning compared with intact, control animals (Landfield et al., 1981). Sapolsky et al. (1985) found that young adult animals, exposed daily to an exogenous corticosterone administration that produced glucocorticoid levels in the upper physiological range, showed profound hippocampal neuron loss following 3 months of treatment. Interestingly, the loss was greatest in corticosterone-sensitive neurons, and the pattern of neuron loss was similar to that occurring in the aged

rat. Therefore, a treatment that mimics the elevated corticosterone levels commonly observed in aged rats is sufficient to produce hippocampal neuron loss, even in young, healthy animals.

It is entirely likely that such hippocampal damage underlies, at least in part, age-related deficits in spatial memory in the rat, because among young animals, hippocampal lesions are consistently associated with these forms of cognitive impairments (e.g., Morris, 1985). However, it is now apparent that not all aged rats manifest spatial memory impairments (e.g., Gage et al., 1984; Gage and Bjorklund, 1986; Pelleymounter et al., 1990). For example, Gage et al. (1984), using the Morris swim maze as a test of spatial memory (Morris, 1985), found that about 30–40% of aged rats showed clearly impaired cognitive performance. About 35% of the aged animals performed as well as young adult animals. These findings reflect the considerable variation in spatial memory ability among aged rats. Such individual differences are also characteristic of human populations and are likely a central feature of CNS aging in mammalian species (see Rowe and Kahn, 1987).

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 animals and aged, cognitively unimpaired animals should differ considerably in HPA activity. In the experiments reported here, we have examined this idea using a screening procedure comparable to that of Gage et al. (1984), selecting animals on the basis of their performance in the Morris swim maze as compared with that of 6-month-old controls. The results indicate that increased HPA activity is indeed selectively associated with progressed hippocampal neuron loss and spatial memory deficits such that aged, cognitively unimpaired animals do not differ from young controls in measures of basal or stress-induced HPA activity.

Materials and Methods

Animals. The aged animals used in these studies were male Long-Evans hooded rats (Charles River Canada, St. Constant, Quebec) obtained at 6–7 months of age. The young controls were obtained from the same supplier at 3–4 months of age. The animals were housed 3 per cage (45 × 30 × 18 cm) and maintained on a 12:12 light–dark schedule (lights on at 0800) at 22°C, 45–55% humidity, with free access to food (Purina Lab Chow) and water. At the time of the experiments, the animals were 6–7 months of age (young controls) or 23–27 months of age (aged). The health of the animals was monitored regularly, and any animals with overt signs of chronic respiratory distress, infection, or tumors were removed from the study. In addition, we maintain a "sentinel" program overseen by the veterinary staff at McGill University. Under this program, specific animals caged in the same location and under the same conditions as our aged animals are blood tested every 3 months and killed at 6 months in order to monitor the health of the animals in our facility and to screen for the presence of viruses or other forms of infection. To date, we have observed a mortality rate of <2% by 24 months and ~10% by 28 months of age.

Behavioral screening. Fifty-eight aged animals were screened using a version of the Morris swim maze test, and their performance was compared to that of 19 young controls. The animals were required to find a platform submerged (2 cm) in a 1.6-m-diameter pool of opaque water using only distal, spatial cues available within the testing room. The platform was hidden in 1 of 4 quadrants halfway between the sidewalls and the center of the pool. Each trial began with the animal placed into the pool facing the sidewalls at either the north, south, east, or west positions in a quasi-random sequence requiring the animals to swim in different directions on successive trials. Each trial ended when the animal found the platform and remained there for 10 sec. If the animal did not find the platform, the trial was terminated after 120 sec. Rats are proficient but reluctant swimmers and readily use the platform to escape the water. The animals were given 12 trials over 3 d with the platform submerged. At the end of the testing period with the platform

in the submerged position, the animals actually selected for the experiments were given 5 trials to locate a platform that was raised 2 cm above the water level (visually cued condition; see Morris, 1985). This condition was used to ensure that impairments were not related to the animals inability to perform the motor demands of the task (see Gage et al., 1984).

Hippocampal cell counting. Cell counting was performed on 20-μm Cresyl violet-stained sections of the dorsal hippocampus as previously described (Meaney et al., 1988a). Briefly, raw cell counts were obtained using a 250 × 250-μm grid over a 40× magnified section with a light microscope. Three to 5 sections of dorsal hippocampus [corresponding to Plates 21 or 22 of the rat brain atlas of Paxinos and Watson (1982)] were analyzed for each animal. Raw cell counts of the pyramidal cell fields were transformed into measures of neuron density per 0.1 mm^2 using the method of Abercrombie (1946) to correct for split-cell artifacts. The medial-lateral length of the cell fields was estimated using camera lucida tracings onto a graphics tablet that provided a measure of the length of each cell field. We also estimated the rostral-to-caudal extent of the dorsal hippocampus in each animal by counting the number of 20μm sections between Plate 19 and Plate 23 of the atlas of Paxinos and Watson. These estimates were made to ensure that differences in neuron density were not due to changes in either the medial-lateral or rostral-caudal dimensions of the cell fields.

Blood sampling under basal and stressful conditions. Basal corticosterone levels over the diurnal cycle were measured as previously described (e.g., Meaney et al., 1989a). Briefly, blood samples (~50 μl) were taken from the tail vein at 6 points over a 24-hr cycle. In these studies, animals were housed in groups of 3 animals per cage, and only 1 animal per cage was actually sampled. Blood samples were taken in heparinized tubes, placed on ice, centrifuged, and stored at −30°C until assayed. Blood samples (250 μl) for ACTH determination were taken at a separate time, 2 hr following the time of lights on (1000, light phase) and off (2200, dark phase) in tubes containing EDTA and Trasylol, centrifuged, stored at −80°C, and assayed for plasma ACTH within 1 week of the experiment. (Note that, because of the larger sample required, sampling for ACTH levels occurs only at 2 time points.) In all cases, animals were sampled less than 10 sec following their removal from their home cages in order to obtain valid estimates of basal hormone levels.

Restraint stress was performed between 1100 and 1300 using tubular, plastic restrainers lined with foam rubber. The animals were placed into the restrainers for a 20-min period. A blood sample (~50 μl) was taken immediately before the animal was placed into the restrainer, and this sample was used as an estimate of basal corticosterone levels prior to stress. Blood samples (~50 μl) were then taken from the same animals at various times during and following the termination of the stressor. The time 0 point was taken just before releasing the animals from the restrainers (i.e., 0 min following termination of the stressor) and represents the peak stress value.

Radioimmunoassays. Plasma corticosterone was measured using the radioimmunoassay of Krey et al. (1975) with a highly specific corticosterone antiserum (B3-163, Endocrine Sciences, Tarzana, CA) and ^{3}H-corticosterone (101.0 μCi/mmol; New England Nuclear, Boston, MA) as tracer. The minimum level of detection with the assay is 10 pg/ml. The antiserum cross-reacts slightly with desoxycorticosterone (~4%), but not with cortisol (<1%). The intra- and interassay coefficients of variation are 8.9 and 11.2%, respectively. Plasma ACTH was measured using the radioimmunoassay kit of ICN-Biomedicals (Carson, CA) with ^{125}I-ACTH as tracer. The ACTH antibody cross-reacts 100% with ACTH$_{1-39}$ and ACTH$_{1-24}$, but less than 1% with β-endorphin, α- and β-MSH, and α- and β-lipotropin. The intra- and interassay coefficients of variation are 6.0 and 10.7%, respectively. The minimal detectable level of plasma ACTH is about 5 pg/ml.

Corticosteroid-receptor binding assays. Animals were killed by decapitation 12–14 hr following adrenalectomy, a time period that allows for the clearance of the endogenous steroid. Following decapitation, the brain was quickly removed and placed on ice, and the hippocampus, hypothalamus, and pituitary were dissected, frozen on dry ice, and stored at −80°C until assayed. The tissue was homogenized in 30 mM Tris, 1 mM EDTA, 10 mM sodium molybdate, 10% v/v glycerol, and 1 mM dithiothreitol (TEDGM) (pH adjusted to 7.4). The homogenate was centrifuged at 2°C for 60 min at 105,000 × g in a Beckman L8-80 ultracentrifuge. For determination of type II glucocorticoid-receptor binding capacity, aliquots (150 μl) of the soluble fraction were incubated with 100-μl aliquots of TEDGM containing a saturating, 15-nM concentration of ^{3}H-RU 28362 (77.9 Ci/mmol; New England Nuclear, Bos-

ton, MA) at 0–4°C for 20–22 hr (a time that has been shown to be sufficient for maximal exchange to occur and during which binding is stable; see Kalimi and Hubbard, 1983; Reul and De Kloet, 1985; Mitchell et al., 1986; Reul et al., 1987; Meaney et al., 1988b). We routinely use exchange conditions in binding experiments in order to minimize the effects of any residual steroid in the tissue. Nonspecific binding was determined in parallel incubations containing a 200-fold excess of unlabeled corticosterone. RU 28362 has been shown to selectively bind to the glucocorticoid receptor, showing very little affinity for the mineralocorticoid receptor (Philibert and Moguilewsky, 1983; Coirini et al., 1985).

Type I mineralocorticoid-like receptor binding capacity in the hippocampus was measured using ^{3}H-corticosterone in a manner previously described by Reul and De Kloet (1985). Aliquots (150 μl) of soluble fractions prepared from hippocampal tissue were incubated at 0–4°C for 20–22 hr in the presence of a saturating 20-nM concentration of ^{3}H-corticosterone (105.0 μCi/mmol; New England Nuclear, Boston, MA) in TEDGM with a 50-fold excess of cold RU 28362 in order to preclude binding of the radioligand to type II sites. Nonspecific binding was determined in parallel incubations containing a 200-fold excess of unlabeled corticosterone.

Sephadex LH-20 columns (4 × 1 cm), made from disposable pipette tips and equilibrated with TEDGM, were used to separate bound from unbound steroids. Following the incubation, 100 μl of the incubates was washed into the columns with 100 μl TEDGM. The columns were eluted 30 min later with 500 μl TEDGM, into minivials, which were then filled with 5 ml of Liquiscent (National Diagnostics, Somerville, NJ) and counted in a Packard scintillation counter at 40% efficiency. Protein content was determined using the method of Bradford (1976), and the results were expressed as fmol/mg protein-specific binding. Protein concentrations ranged from 300 to 500 μg/ml for the hippocampal assays and from 200 to 300 μg/ml for the hypothalamus and pituitary assays.

Plasma corticosteroid-binding globulin (CBG) levels were measured using the method described by Martin et al. (1977) in plasma samples taken either 2 hr into the light cycle (1000) or 2 hr into the dark cycle (2200). Endogenous steroids were removed from plasma samples by passing the sample through a 10 × 1-cm Sephadex LH-20 column. In the process, the plasma was diluted 50:1 with TEDGM (pH, 7.4). Aliquots (225 μl) of the diluted plasma were then incubated in buffer (150 μl) containing a saturating 80-nM concentration of ^{3}H-corticosterone for 90 min at 2–4°C (see Martin et al., 1977). Nonspecific binding was defined in parallel incubations using a 200-fold excess of cold corticosterone. Separation of bound from free protein estimations, the processing of eluates, and the analysis of the data was performed as described above.

Statistical analysis. Unless otherwise stated, the data were analyzed using an analysis of variance with Scheffe post hoc test performed when appropriate.

Results

Behavioral screening of aged animals. The aged animals used in these experiments were selected from a population of 58 rats 23–27 months of age. Overall, the performance of the animals within this group did not vary as a function of age. For the purpose of selecting the animals, the performance of each animal was averaged over blocks of 4 trials, corresponding to each day of testing. The AI group was comprised of animals whose performance on the second and third d of testing (data collapsed over a block of 4 trials) differed significantly (>2 SD) from the mean performance of the young controls (*n* = 19). Animals were considered AU if their performance did not differ from the mean of young controls by more than 0.5 SD on either the second or third day of testing. The data from the first day was not considered as a basis of selection because most animals, regardless of status, tend to perform poorly in the initial phase of the testing. Of the 58 animals tested, 16 (28%) were designated AI, 20 (34%) AU, and 22 fell in between and were excluded from the study. For any subsequent experiment, animals were randomly chosen from these groups.

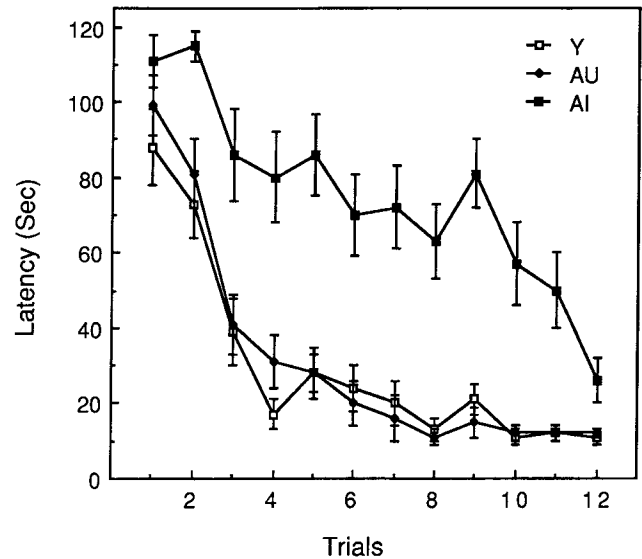Statistical analysis of the latency data for the animals used in

Figure 1. Mean (±SEM) latency to locate submerged platform in Morris swim maze in *AI, AU,* and young control (*Y*) animals over trials (AI differ from AU and Y on trials 3–12, *p* < 0.05).

these experiments revealed that the performance of the AI animals differed significantly (*p* < 0.01) from that of the AU animals and the young controls on trials 3–12 (see Fig. 1). The AU animals and young controls did not differ reliably at any point in testing. The animals were then given 5 trials in which the platform was elevated 1.5 cm above the water level. Under these conditions, there were no significant group differences at any time (all group means < 10 sec). These data suggest that the differences in the ability of the animals to locate the submerged platform was not due to any motor-performance deficits.

Hippocampal neuron density. Four animals from each group were randomly selected for analysis of hippocampal neuron loss (with the subsequent loss of the tissue from 1 AU animal during processing). There were no significant group differences in the length of any of the 4 pyramidal cell fields nor in the rostral-caudal extent of the dorsal hippocampus (see Table 1). The neuron density data were analyzed separately for each of the 4 cell fields of Ammon's horn (see Fig. 2). There was significant hippocampal neuron loss in both AI and AU animals, though the loss was consistently greater in the AI group. The most prominent decrease in neuron density occurred in the CA$_1$ and

Table 1. Mean (±SEM) length of hippocampal cell fields and the rostral-caudal extent of dorsal hippocampus in AI, AU, and young control animals

Measure[a]	AI	AU	Y
CA$_1$ length	9.7 ± 0.2	9.1 ± 0.7	9.6 ± 0.2
CA$_2$ length	2.0 ± 0.2	1.7 ± 0.1	2.3 ± 0.4
CA$_3$ length	5.0 ± 0.2	5.5 ± 0.4	5.6 ± 0.2
CA$_4$ length	5.6 ± 0.1	5.6 ± 0.2	5.6 ± 0.3
Rostral-caudal extent	220 ± 18	223 ± 11	228 ± 10

Y, young control animals; *n* = 3–4 animals per group for all measurements.

[a] Values for CA lengths are expressed as mm ± SEM; values for the rostral-caudal extent are expressed as the number of 20-μm sections.
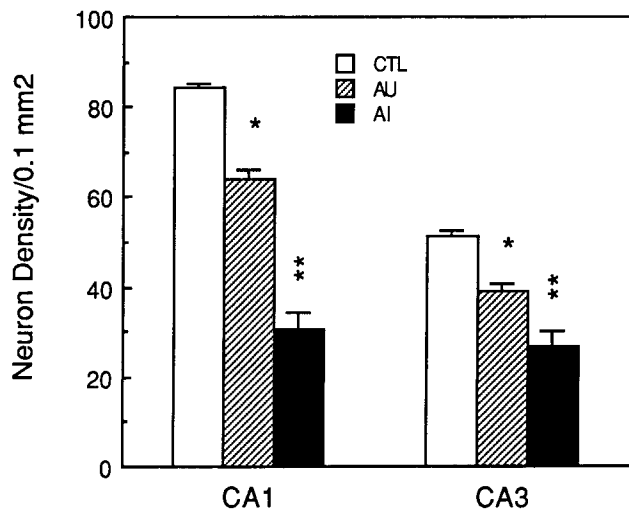
Figure 2. Mean (±SEM) neuron density (per 0.1 mm²) in hippocampal pyramidal cell fields in *AI, AU,* and young control (*CTL*) animals (*n* = 3–4 animals per group; *, *p* < 0.01).

CA$_3$ cell fields (see Fig. 3). In these regions, both AI and AU animals differed from controls (*p* < 0.01), and the AI animals showed significantly (*p* < 0.01) greater decreases in neuron density than did the AU animals. In the CA$_2$ cell field, only the AI animals showed significantly (*p* < 0.05) decreased neuron density compared with the young controls (controls, 49.6 ± 3.1; AU, 48.7 ± 3.0; AI, 35.4 ± 3.8). Likewise, in the CA$_4$ field, the AI animals showed reduced neuron density, but differed significantly (*p* < 0.01) from only the young controls (controls, 40.5 ± 1.8; AU, 27.1 ± 1.7; AI, 21.7 ± 4.3).

Basal and stress-induced HPA function. Basal corticosterone levels (see Fig. 4) showed a significant circadian variation (*p* < 0.001), with the peak in corticosterone levels in all groups occurring at the onset of the dark period (i.e., 2000). There were no significant differences in corticosterone levels at any time between AU and control animals. In contrast, basal corticosterone levels in AI animals were significantly (*p* < 0.01) higher than those in either AU or control animals at 1600, 2400, and 0400. Twenty-four-hour 'secretion' rates, as determined using an area-under-the-curve analysis of the basal samples, revealed that corticosterone levels in AI animals were about 60% higher (*p* < 0.001) than in AU and control animals over the entire diurnal period (controls, 9.5 ± 0.6 μg/dl/hr; AU, 9.3 ± 0.3 μg/dl/hr; AI, 15.1 ± 0.6 μg/dl/hr). Basal plasma ACTH levels showed basically the same pattern (see Fig. 5). There were no significant group differences in the light phase of the cycle; however, during the dark phase, AI animals showed significantly (*p* < 0.01) higher plasma ACTH levels than did either AU or control animals.

Analysis of the stress-induced changes in HPA activity showed that (1) basal prestress corticosterone levels were significantly (*p* < 0.05) higher in the AI animals, (2) there were no group differences in the peak levels of corticosterone achieved during stress (time 0 data), and (3) AI animals had significantly (*p* < 0.01) higher plasma corticosterone levels at each time point following the termination of the stressor (i.e., at 30 min post-stress and thereafter; see Fig. 6). Thus, AI animals were impaired in their capacity to reduce corticosterone levels following the termination of the stressor. This difference can also be illustrated

Table 2. Mean (±SEM) plasma corticosterone-binding globulin levels in AI, AU, and young control animals during the light and dark phases of the cycle

	AI	AU	Y
AM	7.8 ± 1.1	5.9 ± 0.8	7.9 ± 0.6
PM	7.4 ± 1.1	7.6 ± 1.0	6.0 ± 0.6

Values are expressed as mean pmol/mg protein ± SEM; *n* = 3–4 animals per group. Y, young control animals; AM, light phase of cycle; PM, dark phase of cycle.

by estimating the cumulative percentage of animals in each group that showed "recovery" to basal steady–state corticosterone levels following the termination of restraint stress. Recovery is defined as the basal (prestress) value for that animals ±20%. As long as 180 min following the end of restraint stress, only 40% of the AI animals exhibited plasma corticosterone levels that approximated their prestress values, compared with >90% of both AU and control animals.

Corticosteroid-receptor binding. The analysis of the results of the hippocampal costicosteroid-receptor binding assays (see Fig. 7) showed a significant (*p* < 0.05) decrease in type II receptors in both groups of aged animals. The decrease was significantly (*p* < 0.05) greater in the AI animals. The AI animals also showed significantly (*p* < 0.05) reduced hippocampal type I receptor binding capacity. Thus, the overall loss of hippocampal corticosteroid receptors was considerably greater in the AI animals. The groups did not differ in type II receptor binding in the hypothalamus (controls, 132.4 ± 13; AU, 154.4 ± 26; AI, 119.0 ± 11 fmol/mg protein) or in the pituitary (controls, 209.4 ± 55; AU, 165.1 ± 10; AI, 236.7 ± 31 fmol/mg protein). Finally, there were no differences in plasma CBG levels during either the light or dark phases of the cycle (see Table 2). It is important to note that CBG is the principle plasma binder for corticosterone, and Partridge et al. (1983) have shown that the brain uptake of corticosterone approximates the non-CBG-bound portion of the steroid. Thus, the absence of any difference in plasma CBG levels suggests that the percentage of free corticosterone (the biologically active form of the steroid) is comparable across groups, and that the total corticosterone values presented above reflect the actual differences in the magnitude of the corticoid signal in circulation.

Discussion

These data suggest that neither spatial memory impairments nor increased HPA activity are an inevitable consequence of aging in the rat. The behavioral screening revealed evidence of cognitive impairments in only about 30% of the animals tested. In subsequent studies, we have tested about 50 more animals, and the percentage of those classified as aged, impaired remains between 30 and 35%. This estimate is similar to that obtained in the previous studies of Gage (1984) and Gage and Bjorklund (1986). In our animals, spatial memory impairments were associated with a more pronounced loss of hippocampal neurons throughout the pyramidal cell fields: AI animals showed marked decreases in neuron density in all areas examined, whereas the AU animals showed a more modest loss that was detected only in CA$_1$ and CA$_3$. Gage et al. (1984) found that spatial memory impairments in aged rats were selectively associated with decreased hippocampal glucose metabolism. Likewise, Pelleymounter et al. (1988) reported that aged, impaired animals
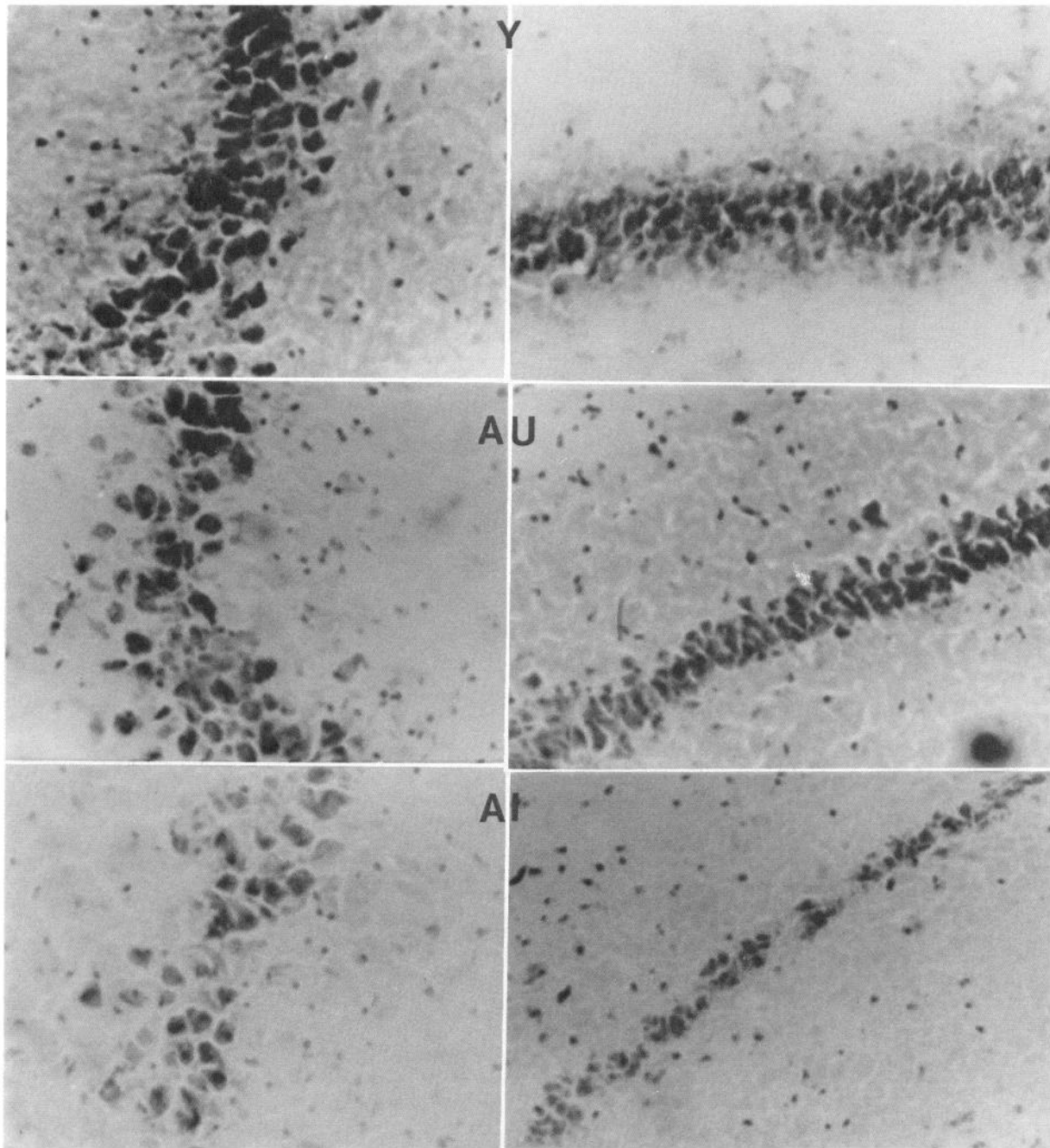
Figure 3. Representative photomicrographs (×500 magnification) of Cresyl violet-stained sections showing the CA₃ (*left panels*) and CA₁ (*right panels*) cell fields in young control (*Y*), *AU,* and *AI* animals.

showed decreased hippocampal NMDA-receptor density compared with both aged, unimpaired animals and young controls. Therefore, spatial memory impairments occur among a select subpopulation of aged rats and appear to be associated with hippocampal pathology. This conclusion is consistent with the finding that hippocampal lesions in young adult rats result in spatial memory deficits (e.g., O'Keefe, 1979; Morris et al., 1982).

The critical question in these studies was whether impaired cognitive performance was selectively related to HPA dysfunction among aged rats. We found that increased HPA activity occurred in the AI, but not the AU animals, and that this difference was apparent under both basal and stressful conditions.

Thus, plasma levels of both ACTH and corticosterone under resting conditions were significantly higher in the AI animals than in either AU or control animals, especially during the dark phase of the cycle. Moreover, while all groups showed comparable increases in plasma corticosterone levels during restraint stress, corticosterone levels remained elevated for a longer period following stress in the AI animals. These findings suggest an impairment in HPA negative-feedback inhibition in the AI animals.

In the present study, both AU and AI animals showed a decrease in type I and type II corticosteroid-receptor binding capacity that was selective to the hippocampus; receptor pop-
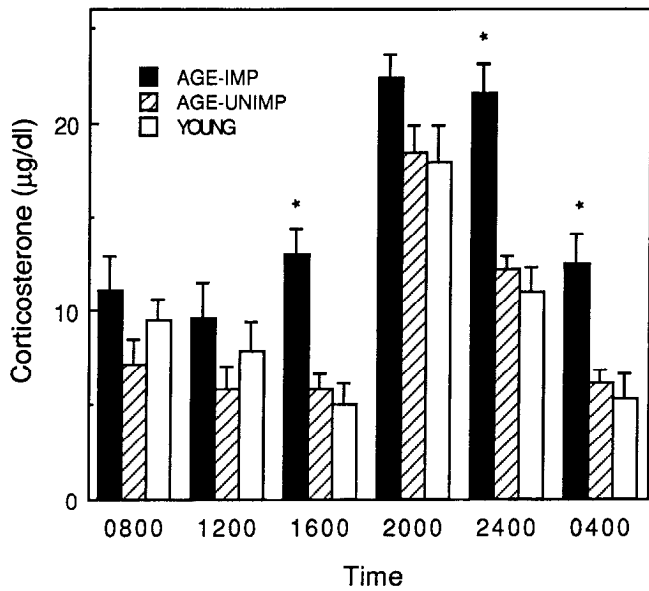
Figure 4. Mean (±SEM) plasma corticosterone levels (μg/dl) in *AI, AU,* and *young* control animals (*n* = 10–12 animals per group) at various times over a 24-hr cycle (*, *p* < 0.05 compared with young controls).

Figure 6. Mean (±SEM) plasma corticosterone levels (μg/dl) in *AI, AU,* and young control (*CTL*) animals (*n* = 10–12 animals per group) at various times before (*PRE*), during (*0 min*), and following (*30–180 min*) a 20-min period of restraint stress (AI differ from AU and CTL animals at 30, 60, 90, 120, and 180 min; *p* < 0.05).

ulations in the hypothalamus and pituitary were unaffected. These data are consistent with several previous studies showing a decrease in corticosteroid receptors in the hippocampus of the aged rat (Sapolsky et al., 1983b; Ritger et al., 1984; Meaney et al., 1988a, 1990a,b; Reul et al., 1988). However, the loss of corticosteroid receptors was significantly greater in the AI group, and this increased hippocampal-receptor loss is probably related to the elevated HPA activity in these animals.

There is now considerable evidence for the importance of the hippocampus in the regulation of HPA activity (for reviews, see McEwen et al., 1986; Sapolsky et al., 1986b; Dallman et al., 1987; De Kloet and Reul, 1987). Hippocampal lesions result in elevated corticosterone levels under basal and poststress conditions (e.g., Feldman and Conforti, 1980; Fischette et al., 1980;
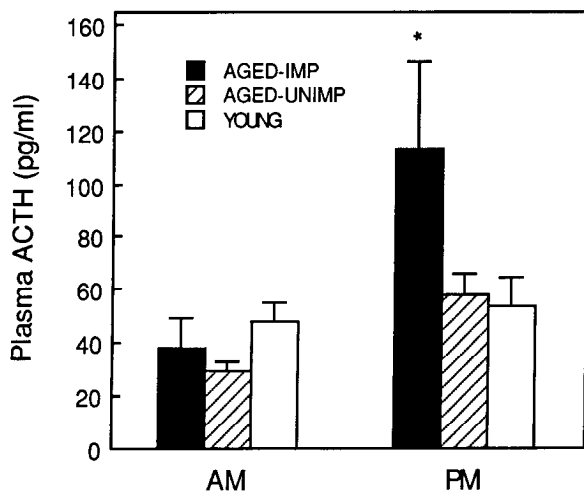
Wilson et al., 1980; Sapolsky et al., 1984a,b) and a reduced suppression of HPA activity following exogenous glucocorticoid administration (Feldman and Conforti, 1976). Hippocampectomy also results in increased CRF and vasopressin mRNA content in the parvocellular region of the paraventricular nucleus (Herman et al., 1989) and increased portal concentrations of ACTH secretagogues (Sapolsky et al., 1989). Moreover, glucocorticoid implants into the hippocampus normalize ACTH levels in adrenalectomized rats (Bradbury and Dallman, 1989). In addition to these anatomical studies, there are also a number of studies showing that decreased hippocampal corticosteroid-receptor density (without cell damage) is consistently associated with glucocorticoid hypersecretion. The Brattleboro rat (Sapolsky et al., 1984a), chronically stressed rats (Sapolsky et al., 1984b), and lactating rats (Meaney et al., 1989b) all show decreased hippocampal corticosteroid-receptor binding, and all hypersecrete corticosterone (Sapolsky et al., 1984a; Stern and Levine, 1974). In the vasopressin-deficit Brattleboro rat, vasopressin therapy reverses the deficit in hippocampal corticosteroid-receptor binding, and as long as the therapy is maintained, corticosterone hypersecretion is alleviated (Sapolsky et al., 1984a).

Hippocampal inhibition of HPA activity appears to involve both type I and type II receptors. Thus, both RU 28318, an

Figure 5. Mean (±SEM) plasma ACTH levels (pg/ml) in *AI, AU,* and *young* control animals (*n* = 10–12 animals per group) 2 hr into the light phase (*AM*) or the dark phase (*PM*; *, *p* < 0.05 compared with young controls).
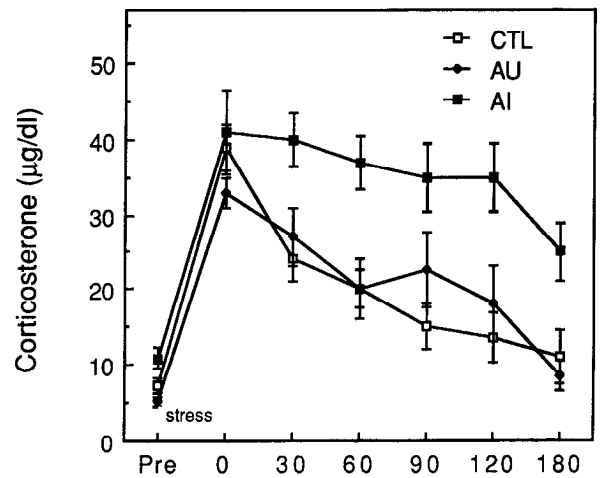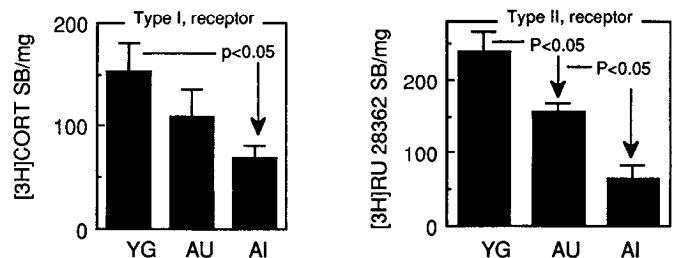

Figure 7. Mean (±SEM) specific binding (fmol/mg protein) for ³H-corticosterone (+cold RU 28362, type I corticosteroid-receptor binding) and ³H-RU 28362 (type II corticosteroid-receptor binding) in *AI, AU,* and young control (*YG*) animals (*n* = 5–6 animals per group; *, *p* < 0.05).

antagonist selective for type I sites, and RU 38486, an antagonist selective for type II sites, increase basal and poststress HPA activity (i.e., antagonize endogenous glucocorticoid negative feedback; Ratka et al., 1989). Portal concentrations of ACTH secretagogues are negatively correlated with occupancy of both type I and type II receptors in the hippocampus (Sapolsky et al., 1990). Finally, both type I and type II receptor agonists suppress ACTH release when implanted directly into the hippocampus (Bradbury and Dallman, 1989). These data suggest that, with age, the loss of receptors decreases the sensitivity of the hippocampus to circulating glucocorticoids and dampens negative-feedback efficacy, and there ensues an increase in circulating corticosterone levels under both basal and poststress conditions. Sapolsky et al. (1986a) have previously documented the decreased negative-feedback sensitivity to elevated corticosterone levels in the aged rat. In the present study, the loss of corticosteroid receptors was greatest in the AI animals, and this finding is consistent with the increased HPA activity in these animals.

The present findings parallel our previous work with aged handled and nonhandled animals. Postnatal handling selectively increases type II corticosteroid-receptor density in the hippocampus (Meaney and Aitken, 1985; Meaney et al., 1985, 1987, 1988a, 1989a, 1990a; Sarrieau et al., 1988). Handled animals also show increased negative-feedback suppression of HPA activity in response to either corticosterone or the synthetic glucocorticoid dexamethasone (Meaney et al., 1989a). With age, there is a decrease in hippocampal corticosteroid-receptor density in both handled and nonhandled rats; however, the handled animals continue to show significantly higher type II receptor density than do the nonhandled animals (Meaney et al., 1990a). This situation is analogous to the receptor difference between AU and AI animals. As nonhandled animals age, they show increased basal levels of both ACTH and corticosterone (Meaney et al., 1988a, 1990b). As in the present study, this effect is largely specific to the dark phase of the cycle. Aged, nonhandled animals also show increased poststress secretion of corticosterone similar to that seen in the AI animals (Meaney et al., 1988a). These findings indicate that the cumulative exposure to circulating glucocorticoids in the aged, nonhandled animal, like the AI animal, is markedly elevated. Aged, nonhandled animals also show significant decreases in hippocampal neuron density and spatial memory impairments. In contrast, the aged, handled animals show only a modest increase in poststress secretion of corticosterone and no increase in basal levels of either ACTH or corticosterone. These animals show virtually no decrease in hippocampal neuron density and no evidence of spatial memory impairments, a scenario that is similar to the AU animals in the present study. Taken together, these findings strongly support the idea that increased HPA activity accounts, in part, for individual differences in the occurrence of age-related hippocampal pathology and cognitive deficits. Thus, HPA dysfunction among aged animals is selectively associated with spatial memory impairments and increased hippocampal neuron loss and does not emerge merely as a function of age.

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