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Prolonged Glucocorticoid Exposure Reduces Hippocampal Neuron Number: Implications for Aging[1]

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Abstract

The hippocampus of the rat loses neurons with age, a loss which may eventuate in some of the functional impairments typical of senescence. Cumulative exposure to corticosterone (CORT) over the lifespan may be a cause of this neuronal loss, as it is prevented by adrenalectomy at mid-age. In this study, we demonstrate that prolonged exposure to CORT accelerates the process of cell loss. Rats were injected daily with sufficient CORT to produce prolonged elevations of circulating titers within the high physiological range. Animals treated for 3 months (chronic subjects) resembled aged rats in a number of ways. First, both groups had extensive and persistent depletions of CORT receptors in the hippocampus; in the case of chronic rats, no recovery of receptor concentrations occurred 4 months after the end of steroid treatment. Second, autoradiographic analysis revealed that the receptor depletion was due, in part, to a loss of CORT-concentrating cells, especially in the CA3 cell field. Remaining cells bound significantly less [^{3}H]corticosterone than did those of control rats. Finally, analysis of size distributions of hippocampal cell bodies indicated that chronic subjects lost neurons of the same size as those lost in the aged hippocampus. Furthermore, chronic subjects also had increased numbers of small, darkly staining cells of CA3; these corresponded in size to the dark glia whose numbers increase in the aged hippocampus, and which are thought to infiltrate in response to neuronal damage or destruction. Thus, this study supports the hypothesis that cumulative exposure to CORT over the lifespan may contribute to age-related loss of neurons in the hippocampus, and that prolonged stress or exposure to CORT accelerates this process.

The hippocampus of the rat progressively loses corticosterone receptors (CORT-Rs) with age (Angelucci et al., 1980; Sapolsky et al., 1983a). A small CORT-R depletion is noted in the amygdala, but not in other brain regions or in the pituitary gland (Sapolsky et al., 1983a). The receptor loss is attributable to decreased concentrations of cytosolic CORT-Rs, with no change in receptor affinity or capacity for cell nuclear accumulation. The loss appears to be limited to neuronal receptors, with no change evident in the concentration of glial CORT-Rs (Sapolsky et al., 1983a), and is most dramatic among pyramidal neurons of the CA3 region (Sapolsky et al., 1984a). Finally, this hippocampal CORT-R depletion appears to be at least partially responsible for the dysfunctions of the adrenocortical axis in the aged rat (Sapolsky et al., 1984b).

We have searched for a means to correct the CORT-R deficit in the aged hippocampus. Alterations in the vasopressin content of the hippocampus have been reported to alter hippocampal CORT-R concentrations (Veldhuis and de Kloet, 1982), and these appear to involve changes in the numbers of receptors per neuron (Sapolsky et al., 1984a). In contrast, the CORT-R depletion in the aged hippocampus appears to involve a loss of steroid-concentrating neurons (Sapolsky et al., 1984a). Thus, we are attempting to uncover the cause of the loss of hippocampal neurons with age.

We have considered the possibility that hippocampal neurons are lost due to cumulative exposure to glucocorticoids. Chronic exposure to stress or to elevated titers of corticosterone (CORT) down-regulates CORT-Rs in an anatomical pattern identical to that seen in the aged rat (Sapolsky et al., 1984c). Furthermore, pharmacologic dosages of glucocorticoids reduce hippocampal neuron number (Aus Der Muhlen and Ockenfels, 1969). Finally, basal titers of CORT progressively rise with age (Landfield et al., 1978; Tang and Phillips, 1978; Sapolsky et al., 1983b; De Kosky et al., 1984), and adrenalectomy at 12 months can prevent the age-related loss of hippocampal neurons (Landfield, et al., 1981a). Thus, we designed the present studies to determine whether prolonged exposure to titers of CORT in the high physiological range would eventuate in the loss of CORT-concentrating neurons characteristic of the aged hippocampus. We present evidence for such a phenomenon and, in the following paper (Sapolsky, 1985), examine possible mechanisms by which glucocorticoids can be toxic to hippocampal neurons.

Materials and Methods

Subjects and treatments. Male Fischer 344 strain rats (Charles River Breeding Farms, Wilmington, Delaware) were utilized. Subjects were Caesarian-delivered and housed in pathogen-free colonies. All rats were given access to food and water *ad libitum* and were maintained on a 14-/10-hr light/dark cycle (lights on: 6:00 A. M. to 8:00 P. M.). Subjects were injected subcutaneously daily with 5 mg of CORT (Sigma Chemical Co., St. Louis, MO) in 1 ml of sesame oil. A single injection produced prolonged (minimum of 8 hr) elevations in circulating titers of CORT equivalent to those seen during stress (Table I). Short-term subjects (acute) received daily injections for 2 weeks. Long-term subjects (chronic) received injections for 3 months. Injections were terminated at that time because of a 50% mortality rate. Control subjects (CONT) received no CORT. All subjects were 8 months old at termination of treatment. Subjects were then used either for high resolution autoradiography of hippocampal CORT-Rs or for assays of hippocampal cytosolic CORT-Rs at various times following the end of treatment. Autoradiography was also conducted on untreated 28-month-old subjects, for use in comparisons with acute and chronic subjects.

Cytosolic CORT-R assays. Receptor concentrations were assayed either 2 days, 1 or 2 weeks, or 1, 2, or 4 months after the end of CORT treatment. Subjects were adrenalectomized and decapitated 12 hr later. This time interval was used, rather than a 24-hour interval, because of the fragile health of some of the chronic subjects. Pairs of hippocampi (dorsal, ventral, and subiculum) were pooled and homogenized in 5 mM Tris buffer (pH 7.4)

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TABLE I

Efficacy of CORT injection in producing prolonged elevations of titers of CORT equivalent to stress-induced titers of the steroid (micrograms per 100 ml)

Treatment	Basal	Stressed	Rise
Cage transfer (30 min after transfer)	8.1 ± 1.6	15.2 ± 3.1	+7.1
Cold exposure (30 min into exposure to 4°C)	7.2 ± 1.3	15.4 ± 3.6	+8.2
Ether exposure (20 min after a 1-min exposure)	3.9 ± 0.9	22.3 ± 2.9	+18.4
Histamine injection (20 min after i.v. injection of 1 mg)	5.7 ± 2.6	15.8 ± 2.9	+10.1
CORT injection (5 mg in sesame oil) of adrenalectomized subjects			
0 hr (pre-injection)			<1 μg
4 hr			17.2
8 hr			12.8
24 hr			1.9

containing 1 mM EDTA, 10 mM sodium molybdate, 10% glycerol, and 1 mM dithiothreitol. The homogenate was centrifuged at 1°C for 30 min at 105,000 × *g*. Aliquots (250 μl) of cytosol were added to solutions containing various concentrations of [^{3}H]dexamethasone (5 to 40 nM, as determined by direct counting of aliquots). Nonspecific binding was determined in parallel incubations which also contained a 500-fold excess of unlabeled CORT. Macromolecular bound steroid was isolated by chromatography on LH-20 columns, and eluates were counted in Liquiscint (National Diagnostics, Somerville, NJ) at an efficiency of 35%. Cytosol protein concentrations were determined (Lowry et al., 1951) using bovine serum albumin (BSA) as a standard, and results were standardized as femtomoles of [^{3}H]dexamethasone bound per milligram of cytosolic protein. Maximum binding (B_{max}) and K_d were derived by the method of Scatchard (1949).

Radioimmunoassay of corticosterone. Sera were separated after centrifugation and assayed for CORT by radioimmunoassay (Krey et al., 1975) using an antiserum generated against cortisol 21-succinate BSA (antisera F21-53, Endocrine Sciences, Tarzana, CA). This antiserum cross-reacts with CORT (~ 60%) but not with progesterone. In the present studies, [1,2,6,7-^{3}H]cortisol (Amersham, Arlington Heights, IL) was used as the tracer. Assay sensitivity was 20 pg of CORT, and coefficients of variation within and between assays were 0.07 and 0.10, respectively (n = 8).

High resolution autoradiography and morphological analysis. Autoradiography was conducted according to the method of Gerlach and McEwen (1972). Subjects were adrenalectomized, injected subcutaneously 10 hr later with 100 μCi of [^{3}H]CORT, and decapitated 2 hr later. Frozen, 6-μm-thick sections were cut with a cryostat at −18°C and transferred with a fine brush onto emulsion-dipped slides. Slices were taken every 75 μm through the hippocampus. Slides were desiccated and stored in the dark at 4°C for 12 months before being developed. Slides were then stained with cresyl violet for anatomical reference, using the anatomical criteria of Lorente de No (1934) for identification of hippocampal cell fields.

The number of CORT-concentrating neurons and the number of grains concentrated per neuron were determined in each slice by microscopic examinaton (× 40 power). After orienting at the center of a particular cell field, the number of neurons within the field of a 400 × 400 μm ocular grid was counted. The average size of neurons was assessed by dividing the number of ocular grids overlapped by cells by the number of cells; average background grain density in neuropil was then determined for an area equivalent to the average neuron size. With this information, we then determined the total number of neurons within view and the total number which concentrated grains at a rate higher than background. Twenty such cells which concentrated CORT were randomly chosen per cell field per slice, and the precise number of grains (above background) was counted.

Distribution of cell area was determined by orienting at the boundary between CA2 and CA3 of the hippocampus. Moving through CA3 from the boundary, the outlines of all cells containing nuclei encountered within the cell layer were traced onto a digitizing tablet (Summagraphics) which had a Megatek vector graphics display unit. The microscope was used in conjunction with a camera lucida drawing tube. The area of each cell was then determined with an Area Program of a PDP 1134 computer (West et al., 1976). (We thank Joyce Powzyk and Jane Sekulski of the Torsten Wiesel Laboratory for use of this system.) Tracings were made at × 100. One hundred cells per unilateral hippocampal CA3 cell field per slice were traced. The total length of CA3 center field covered in the course of encountering 100 discrete cells was noted, so that cell area information could be converted to measures per unit length of cell field. Two slices from dorsal hippocampus per animal were analyzed.

With this information, histograms were constructed of occurrences of cells of varying areas. Because larger nuclei are more likely to be sectioned and counted than smaller nuclei, thus introducing a counting error for cells in different area categories, it was necessry to correct for this split nuclei artifact. The correction equation of Abercrombie (1946) was used, after determining the mean diameter of nuclei of cells in each area category. Mean diameters were found not to differ significantly between acute and chronic subjects (data not shown).

Determination of hippocampal volume. Cresyl violet-stained sections of hippocampus were projected (× 16) onto a digitized screen, and the area of each section was calculated using the computer program described above. Similar determinations were made for the areas of individual cell fields at × 40.

Statistics. Data were analyzed by one-way and two-way analysis of variance followed by Scheffe post hoc tests.

Results

Prolonged exposure to titers of CORT in the high physiological range seen during stress reduced the CORT-R number in the hippocampus, in agreement with previous reports (Sapolsky et al., 1984c). Furthermore, there appeared to be a limit to the extent of down-regulation possible, as acute (2 weeks) and chronic (3 months) subjects showed comparable decreases in CORT-Rs (Fig. 1). Approximately 55% reductions were observed. We have previously shown no change in affinity of the receptor with such treatment (Sapolsky et al., 1984c).

We next examined whether CORT-R concentrations recovered to control levels following the cessation of CORT treatment (Fig. 1). The substantial declines in CORT-R number in acute subjects had been reversed during 1 week of recovery. Receptor concentrations were then comparable to those of control rats and did not change thereafter. In contrast, chronic subjects showed no evidence of recovery of CORT-Rs and had significant depletions of receptors 4 months after the end of treatment.

We then examined the patterns of CORT-Rs in the hippocampus with high resolution autoradiography. Representative autoradiograms of the CA3 cell field for control, acute, chronic, and aged subjects are presented (Fig. 2, *A* to *D*). We found differences in the cellular bases of the CORT-R depletions between acute and chronic subjects which could explain the seeming irreversibility of the receptor loss in the latter group. Specifically, acute subjects had a reduction in the number of CORT-Rs per cell, without a change in the total number of cells. Chronic subjects, in contrast, had a loss of both receptors per cell, and of the steroid-concentrating cells.

Neuron loss was concentrated in the CA3 cell field (Table II). Acute and control subjects had an equivalent number of cells per unit cell field. Chronic subjects, however, had a significant reduction in the total number of cells. Cells which did not bind CORT did not change in number. Rather, the loss of cells was entirely attributable to a loss in the number of steroid-concentrating cells. No such change occurred in acute subjects. In both acute and chronic animals, cells that concentrated steroid above background accumulated significantly fewer grains, indicating decreased numbers of receptors per cell.

A somewhat different pattern was found in other parts of the hippocampus. In the CA1 cell field, for example (Table II), acute and chronic subjects did not differ from each other. Neither had a loss in the total number of cells per unit length of cell field. Both had a significant reduction in the number of specifically labeled cells and an equivalent increase in the number of unlabeled cells, indicating that the CORT treatment had down-regulated CORT-R number in many cells to background concentrations. Both groups had significant reductions in the number of grains per labeled cell.

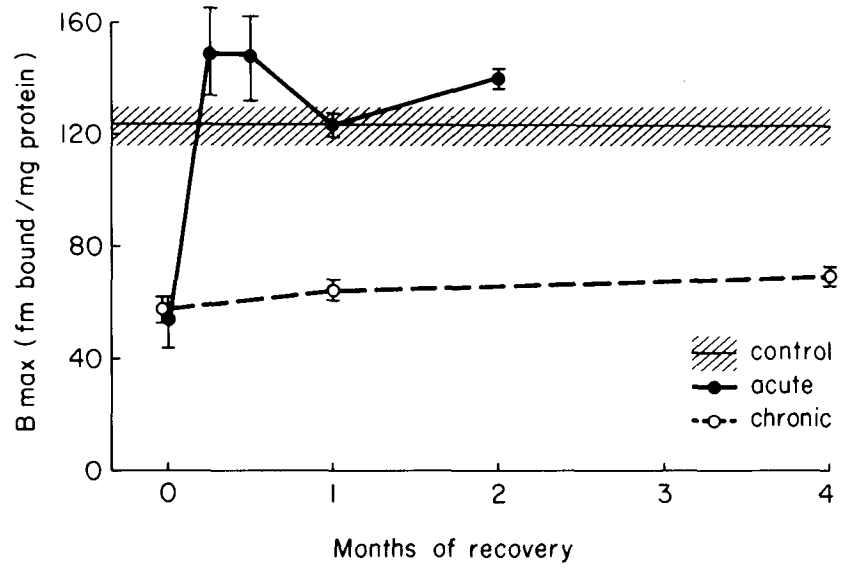

Figure 1. Maximal binding capacity (in femtomoles of [^{3}H]dexamethasone bound per milligram of cytosolic protein) of hippocampi of control, acute, and chronic subjects. Acute subjects were injected subcutaneously daily with 5 mg of CORT for 2 weeks, and chronic subjects were injected daily for 3 months. Subjects were then allowed to recover from such treatment; four subjects from each group were culled at each time point during the recovery period for receptor assay. Both acute and chronic subjects had significantly diminished binding capacity, relative to controls, at the beginning of the recovery period (0.01 level of significance, Scheffe test following two-way analysis of variance). Acute subjects did not differ from controls thereafter, whereas chronic subjects presented equivalently significant depletions of binding capacity throughout the remainder of the recovery period.

Thus, CORT treatment caused reductions in the numbers of CORT-Rs in both groups in CA1 as well as in CA3. In addition, there was a decreased density of cells in the CA3 cell field of chronic subjects, due to a loss of steroid-concentrating cells. Table III demonstrates that acute and chronic rats did not differ in the total volume of the hippocampus or in the volume of specific cell fields. Therefore, the decreased density of cells per unit length of CA3 in the chronic subjects implies a reduction in the absolute number of cells.

We next determined the size distribution of cells in the CA3 region, in order to understand further the nature of the cell loss. Control subjects had cell bodies with cross-sectional areas ranging from 28 to 374 μm^2 (Fig. 3), with a mean area of 136 μm^2. Acute subjects had an area distribution which did not differ significantly from that of control subjects. Chronic subjects, however, had a significant reduction in the number of cells in the 140- to 280-μm^2 range, and cells of this size now comprised a significantly smaller percentage of the total cell size distribution. Chronic subjects also had a significant increase in the number of cells in the smallest size category, 0 to 70 μm^2. The increase in cell number in this size category did not offset the loss of the larger cells, and chronic subjects had a loss in the overall number of cells, in agreement with Table II. This shift in the size distribution of CA3 cells in chronic subjects was quite similar to that seen in aged rats. The total number of cells declines in CA3 of aged rats (Landfield et al., 1981a; Sapolsky et al., 1984a), and this was due to the loss of larger cells (140- to 280-μm^2 range; Fig. 3). As with chronic subjects, this was accompanied by a significant increase in the number of small (0 to 70 μm^2) cells in the CA3 field.

In the photomicrograph in Figure 2*D*, an *arrow* indicates a representative cell of the 0- to 70-μm^2 size class (which increases in number in chronic and aged subjects); an *arrow* pointing to an *asterisk* indicates a representative cell of the 140- to 280-μm^2 size class (which decreases in number in those same subjects).

Discussion

As it ages, the hippocampus and overlying cortex sustain a progressive loss of neurons, as judged by histologic, morphologic and cytologic criteria (Landfield et al., 1977, 1981a; Brizzee and Ordy, 1979). There are also indications of increased numbers of microglia and dark oligodendroglia with age (Ling and Leblond, 1973; Vaughan and Peters, 1974; Landfield et al., 1981b). The glia are typically clustered, and they are likely to have infiltrated in response to neuron damage or death (Wisniewski and Terry, 1973; Landfield et al., 1981b). This glial hyperplasia is reflected in increased numbers of glial CORT-Rs in the aged hippocampus, which is inferred from increased uptake of [^{3}H]dexamethasone *in vivo* (Sapolsky et al., 1983a). The neuronal loss and glial hyperplasia in the aged hippocampus are well demonstrated in Figures 2 and 3, where the morphologic and size characteristics of the large cells which are lost are those of neurons, whereas the small, darkly staining cells whose numbers increase resemble dark glia.

The aging hippocampus also undergoes a progressive loss of CORT-Rs (Angelucci et al., 1980; Sapolsky et al., 1983a), a decline which eventuates in functional impairments in the adrenocortical stress response (Sapolsky et al., 1984b). This loss is limited to neuronal CORT-Rs (in contrast to the inferred increased numbers of glial CORT-Rs; Sapolsky et al., 1983a) and is most pronounced in the CA3 cell field (Sapolsky et al., 1984a). We have previously demonstrated that the neurons which are lost with age in the hippocampus include those containing CORT-Rs. This cell loss accounts for the receptor loss as well as its relative pharmacological intractability to treatments designed to reverse receptor loss (Sapolsky et al., 1984a).

We hypothesized that the loss of neurons and of their CORT-Rs is due to the cumulative exposure to CORT over the animal's lifespan. This is suggested by the aforementioned vulnerability of neurons containing CORT-Rs. Furthermore, pharmacologic dosages of glucocorticoids destroy hippocampal neurons (Aus Der Muhlen and Ockenfels, 1969). Finally, basal CORT titers rise progressively with age (Landfield et al., 1978; Tang and Phillips, 1978; Sapolsky et al., 1983b; De Kosky et al., 1984); adrenalectomy at middle-age prevents the age-related loss of hippocampal neurons (Landfield et al., 1981a).

In the present study, we have tested the hypothesized linkage between corticosterone and cell loss, examining whether sustained exposure to high physiological titers of CORT produces changes similar to those seen during aging; i.e., prolonged CORT-R losses, loss of receptor-bearing neurons, and glial hypertrophy in those regions of neuron loss. We found that 2-week exposure to CORT was not an appropriate model for aging because the effects were reversible. CORT-R number was markedly reduced in such acutely treated rats (involving reductions in total cellular receptor pools, in addition to cytosolic concentrations, and not due to residual contamination with injected steroids; Sapolsky et al., 1984c). However, within a week after the end of CORT treatment, the concentration of receptors recovered to normal, and the receptor loss was entirely attributable to a loss of receptors per cell, as shown by the decreased numbers of grains per cell in autoradiograms. The decreased numbers of labeled cells and the equivalent increase in unlabeled ones indicates that in some parts of the hippocampus, down-regulation reduced binding to background levels. This rapid

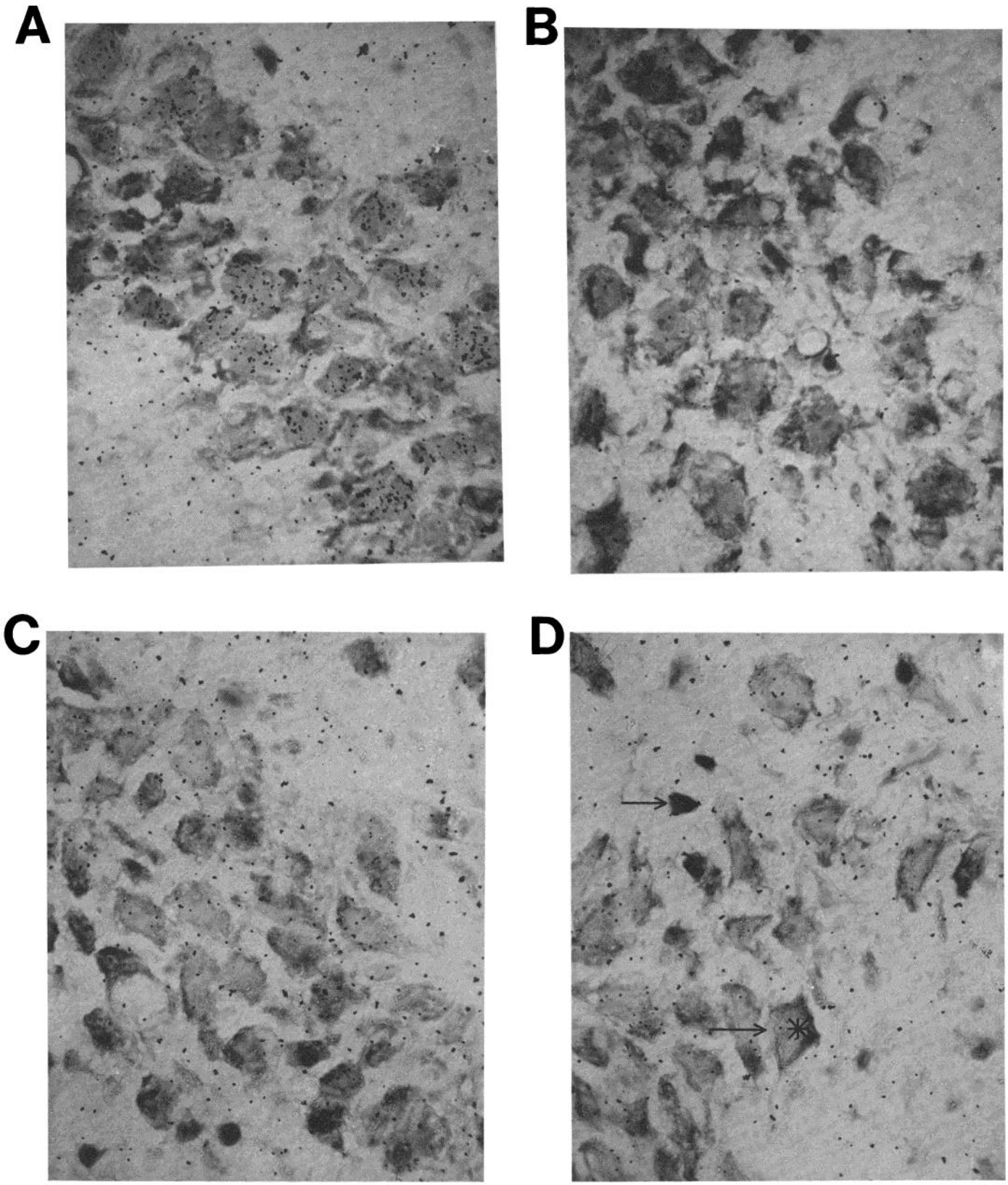

Figure 2. Representative photomicrographs of autoradiograms of the CA3a pyramidal cell region of hippocampus in control (*A*), acute (*B*), chronic (*C*), and aged (*D*) subjects. The *upper arrow* in *D* indicates a representative cell of the 0- to 70-μm^2 size class; the *arrow* pointing to the *asterisk* indicates a representative cell of the 140- to 280-μm^2 size class. Magnification × 400.

TABLE II

Effects of CORT treatment on the numbers and CORT-binding characteristics of hippocampal cells in areas CA3 and CA1

Total cell number indicates the number of cells within a field overlapped by an ocular grid at × 40 power. Number of cells labeled indicates the number concentrating CORT above background. Number of grains/labeled cell involves analysis only of cells concentrating CORT above background. For each group, $n = 3$. Values are the mean ± SE. Clusters of grains were estimated as ranging from two to four individual grains. See the text for more detailed methods. Acute subjects were treated daily with 5 mg of CORT for 2 weeks; Chronic subjects were similarly treated or 3 months.

Area	Control	Acute	Chronic
CA3			
Total cell number	69 ± 3	67 ± 14	52 ± 4[a]
Number of cells labeled	25 ± 3 (36%)[b]	18 ± 5 (25%)	8 ± 1 (13%)[c]
Number unlabeled	44 ± 2	49 ± 9	44 ± 4
Number grains/labeled cell	9.9 ± 1.5	6.3 ± 0.5[a]	4.5 ± 2[a]
CA1			
Total cell number	52 ± 4	53 ± 1	48 ± 1
Number of cells labeled	28 ± 3 (53%)	15 ± 2.5 (27%)[c]	10 ± 1 (29%)[c]
Number unlabeled	24 ± 3	38 ± 1.5[c]	38 ± 1[c]
Number grains/labeled cell	9.6 ± 1	4.8 ± 1[a]	3.1 ± 1[a]

[a] $p < 0.05$ (two-tailed Scheffe comparison of treated groups with controls after one-way analysis of variance).

[b] Numbers in parentheses, percentage of total cells which were labeled.

[c] $p < 0.01$ (two-tailed Scheffe comparison of treated groups with controls after one-way analysis of variance).

TABLE III

Influence of acute and chronic CORT treatments on the length and volume of hippocampus and individual hippocampal cell fields

For each treatment group, $n = 3$. For area determinations, three slices were measured per subject. The anterior coronal sections were taken from a range of 4.3 to 4.6 mm anterior of lambda suture (dorsal-ventral coordinate of lambda set equal to bregma). The medial sections and those for the determination of individual cell field areas were taken from a range of 3.4 to 4.1 mm anterior of lambda. The posterior sections were taken from a range of 1.1 to 2.5 mm anterior of lambda. No significant differences occurred between acute and chronic subjects (*t* test).

	Acute	Chronic
Anterior/posterior extent of hippocampus (mm)	3.72 ± 0.15	3.68 ± 0.11
Area of coronal sections of hippocampus (μm^2)		
Anterior	5.6 ± 0.8	5.9 ± 0.7
Medial	7.7 ± 0.3	8.5 ± 0.3
Posterior	14.4 ± 1.8	11.9 ± 1.0
Area of individual hippocampal cell fields, in coronal section (μm^2)		
CA3	0.203 ± 0.039	0.244 ± 0.047
Dentate gyrus	0.350 ± 0.028	0.397 ± 0.059

and reversible form of receptor autoregulation probably represents altered rates of receptor synthesis or degradation (Tornello et al., 1982; Felt et al., 1984).

Prolonged exposure to CORT for 3 months, however, was an appropriate model for aging. Down-regulation of hippocampal CORT-Rs occurred (to an extent equivalent to that seen in acute subjects, a previously noted "floor effect" (Sapolsky et al., 1984c)), and no recovery of receptor concentration was noted 4 months after the end of CORT treatment. Of prime importance, this persistent receptor loss appears due to a similar cellular mechanism that accounts for the receptor loss in aged rats. In both cases, there were decreased numbers of steroid-concentrating cells, as well as the decreased numbers of receptors per cell seen in all experimental groups. The cell loss was observed in the CA3 cell field but not CA1 in chronic subjects (Table II), and in CA3 and CA1 of aged subjects, but not CA4 (Sapolsky et al., 1984a). We have concluded that the decreased density of cells observed in these regions represents absolute decreases in the total numbers of cells for a number of reasons. First, the overall volume of the hippocampus and the relevant cell fields does not increase in chronic subjects. Second, were the decreased density due to subtly increased volume and subsequent dilution of cells, a decrease would be noted in all cell fields and in all cell sizes. This is not the case. Instead, chronic subjects have selective losses of large hippocampal cells, of similar appearance and size to the neurons lost in the aged hippocampus (Landfield et al., 1977, 1981a; Brizzee and Ordy, 1979). Whereas it is conceivable, although unlikely, that there is a loss of smaller neurons coupled with shrinking of larger ones, the most parsimonious conclusion is that chronic subjects have losses in the same size class of neurons as do aged rats. In addition, chronic subjects have increases in the numbers of small cells, which have staining characteristics and size similar to those of the dark glia whose numbers increase in the aged hippocampus (Ling and Leblond, 1973; Vaughan and Peters, 1974; Landfield et al., 1981b). Such invasive

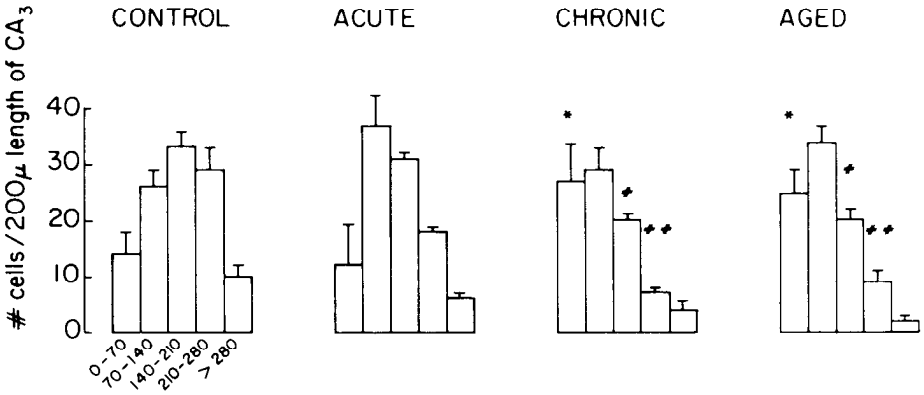

Figure 3. Histograms of the distribution of cell body sizes in the CA3 pyramidal cell region of hippocampi of control, acute, chronic, and aged subjects. The area of all cell bodies in the first 200 μm of the CA3 region (moving from the CA2/CA3a boundary) containing nuclei was determined as described under "Materials and Methods." Cell body areas were then grouped into the categories of 0 to 70, 70 to 140, 140 to 210, 210 to 280, and >280 μm^2 of area. Values are mean ± SE, $n = 3$ per experimental group. *, significantly greater number of cells, relative to controls, at 0.05 level of significance; and, significantly fewer cells, relative to controls, at 0.05 and 0.01 levels of significance, respectively (Scheffe test following two-way analysis of variance).

and clustered glia are typical markers of neuronal damage or death, both during aging and following brain injury (Wisniewski and Terry, 1973; Scheibel and Scheibel, 1975).

In summary, striking similarities occur between hippocampi of aged rats and those of rats exposed to prolonged and elevated titers of CORT. In addition to loss of receptors per cell (as was also seen in acute subjects), both display persistent depletions of CORT-Rs due to loss of CORT-concentrating cells (in contrast to acute subjects). This cellular loss is most dramatic in the CA3 region, and the cells that are lost are of similar size. Finally, both show the increased numbers of darkly staining glia typically found after brain tissue damage. These findings support and extend the previous suggestions of Landfield et al. (1981a), that the loss of hippocampal neurons with age is due to cumulative exposure to increasingly elevated titers of CORT. In the following paper (Sapolsky, 1985), we examine the effects of CORT on kainic acid- or 3-acetylpyridine-induced hippocampal neuronal loss in order to obtain insights into the mechanisms underlying CORT neurotoxicity.

References

Abercrombie, M. (1946) Estimation of nuclear populations from microtome sections. Anat. Rec. *94:* 239–247.

Angelucci, L., P. Valeri, E. Grossi, H. Veldhuis, B. Bohus, and E. De Kloet (1980), Involvement of hippocampal corticosterone receptors in behavioral phenomena. In *Progress in Psychoneuroendocrinology*, F. Brambilla, G. Racagani, and D. de Wied, eds., pp. 177–185, Elsevier Biomedical Press, Amsterdam.

Aus Der Muhlen, K., and H. Ockenfels (1969) Morphologische veranderungen im diencephalon und telencephalon nach storungen des regelkreises adenohypophyse-nebennierenrinde. III. Ergebnisse beim meerschweinchen nach verabreichung von cortison und hydrocortison. Z. Zellforsch. *93:* 126–141.

Brizzee, K., and J. Ordy (1979) Age pigments, cell loss and hippocampal function. Mech. Ageing Dev. *9:* 143–162.

De Kosky, S., S. Scheff, and C. Cotman (1984) Elevated corticosterone levels: A possible cause of reduced axon sprouting in aged animals. Neuroendocrinology *38:* 33–38.

Felt B., R. Sapolsky, and B. McEwen (1984) Regulation of hippocampal corticosterone receptors by a vasopressin analogue. Peptides, *5:* 1225–1227.

Gerlach, J., and B. McEwen (1972) Rat brain binds adrenal steroid hormone: Radioautography of hippocampus with corticosterone. Science *175:* 1133–1136.

Krey L., K. Lu, W. Butler, J. Hotchkiss, F. Piva, and E. Knobil (1975) Surgical disconnection of the medial basal hypothalamus and pituitary function in the rhesus monkey. II. GH and cortisol secretion. Endocrinology *96:* 1088–1096.

Landfield, P., G. Rose, L. Sandles, T. Wohlstadter, and G. Lynch (1977) Patterns of astroglial hypertrophy and neuronal degeneration in the hippocampus of aged, memory-deficient rats. J. Geront. *32:* 3–12.

Landfield, P., J. Waymire, and G. Lynch (1978) Hippocampal aging and adrenocorticoids: Quantitative correlations. Science *202:* 1098–1102.

Landfield, P., R. Baskin, and T. Pitler (1981a) Brain aging correlates: Retardation by hormonal-pharmacological treatments. Science *214:* 581–584.

Landfield, P., L. Braun, T. Pitler, J. Lindsey, and G. Lynch (1981b) Hippocampal aging in rats. A morphometric study of multiple variables in semithin sections. Neurobiol. Aging *2:* 265–275.

Ling, E., and C. Leblond (1973) Investigation of glial cells in semithin sections. II. Variations with age in the numbers of the various glial cell types in rat cortex and corpus callosum. J. Comp. Neurol. *149:* 73–82.

Lorente de No, R. (1934) Studies on the structure of the cerebral cortex. II. Continuation of the study of the ammonic system. J. Psychol. Neurol. *46:* 113–177.

Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall (1951) Protein measurement with the Folin phenol reagent. J. Biol. Chem. *193:* 265–275.

Sapolsky, R., L. Krey, and B. McEwen (1983a) Corticosterone receptors decline in a site-specific manner in the aged rat brain. Brain Res. *289:* 235–240.

Sapolsky, R., L. Krey, and B. McEwen (1983b) The adrenocortical stress-response in the aged male rat: Impairment of recovery from stress. Exp. Gerontol. *18:* 55–64.

Sapolsky, R. M. (1985) Mechanism for glucocorticoid toxicity in the hippocampus: Increased neuronal vulnerability to metabolic insults. J. Neurosci. *5:* 1227–1231.

Sapolsky, R., L. Krey, B. McEwen, and T. Rainbow (1984a) Do vasopressin-related peptides induce hippocampal corticosterone receptors? Implications for aging. J. Neurosci. *4:* 1479–1485.

Sapolsky, R., L. Krey, and B. McEwen (1984b) Glucocorticoid-sensitive hippocampal neurons are involved in terminating the adrenocortical stress-response. Proc. Nat. Acad. Sci. U. S. A. *81:* 6174–6177.

Sapolsky, R., L. Krey, and B. McEwen (1984c) Stress down-regulates corticosterone receptors in a site-specific manner in the brain. Endocrinology *114:* 287–292.

Scatchard, G. (1949) The attractions of proteins for smaller molecules and ions. Ann. N. Y. Acad. Sci. *51:* 660–703.

Scheibel, M., and A. Scheibel (1975) Structural changes in the aging brain. In *Aging*, H. Brody, D. Harmon, and J. Ordy, eds., Vol. 1, pp. 11–37, Raven Press, New York.

Tang, G., and R. Phillips (1978) Some age-related changes in pituitary-adrenal function in the male laboratory rat. J. Gerontol. *33:* 377–382.

Tornello, S., E. Orti, A. DeNicola, T. Rainbow, and B. McEwen (1982) Regulation of glucocorticoid receptor in the brain by corticosterone treatment of adrenalectomized rats. Neuroendocrinology *35:* 411–419.

Vaughan, D., and A. Peters (1974) Neuroglial cells in the cerebral cortex of rats from young adulthood to old age: An electron microscope study. J. Neurocytol. *3:* 405–439.

Veldhuis, H., and E. de Kloet (1982) Vasopressin-related peptides increase the hippocampal corticosterone receptor capacity of diabetes insipidus (Brattleboro) rats. Endocrinology *110:* 153–157.

West, M., G. Danscher, and S. Laurberg (1976) A determination of the volumes of the rat hippocampal subfields. Soc. Neurosci. Abstr. *2:* 214.

Wisniewski, H., and R. Terry (1973) Morphology of the aging brain, human and animal. Prog. Brain Res. *40:* 167–186.