take of residual components of the transmission process. The more frequent presence of SSPP's might indicate increased synaptic efficacy or use (*13*), or they could indicate greater numbers of a particular kind of synapse that tends to exhibit this morphological specialization. The presence of greater numbers of such synapses in the more experienced groups leads us to suggest that SSPP's may be associated with positive changes in synaptic function, but we must also consider that such changes might represent decreased synaptic efficacy. Finally, it is also possible that SSPP's might indicate differences in the status rather than in the strength of synapses. An SSPP could, for example, be associated with synapse formation (or degeneration), particularly since Golgi staining studies have indicated increasing numbers of spines (believed to indicate synapses) on some types of neurons with increasing rearing environment complexity and with age (*14*). An SSPP might even signify the permanence of a synapse with respect to a preprogrammed removal process affecting unused connections (*15*). In any case, the effect of the organism's experience on the frequency of SSPP's suggests the possible involvement of such changes in neuronal function.

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5. The rats were anesthetized with sodium pentobarbital and perfused intracardially with 15 ml of 0.54 percent dextrose in 0.1*M* sodium phosphate buffer (*p*H 7.4) followed by 150 ml of 0.5 percent glutaraldehyde and 4 percent paraformaldehyde in the same buffer-dextrose solution. A 1-mm square column of occipital cortex, 2 mm lateral to the midline and 2 mm anterior to the posterior pole, was dissected and placed in cold perfusate for 1/2 to 1 hour, postfixed in cold 2 percent OsO_4 in buffer-dextrose for 2 hours, rinsed for 1/2 hour in buffer-dextrose, dehydrated through alcohols and propylene oxide, and embedded in Epon [R. W. Guillery, *Am. J. Anat.* **120**, 583 (1967)].
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7. Micrographs were taken at random from the central 75 to 80 percent of the layer (as determined from adjacent light microscopic sections), the only constraint being that cell bodies were excluded.
8. The group difference could also reflect existing SSPP's growing larger and hence being more frequently sampled in transverse sections. However, the average measured size of SSPP's did not differ statistically across groups (EC = 3.9, SC = 4.2, IC = 3.6, d.f. = 2, 552; $F = 2.44$; $P > .05$), as it would have if they were larger in the more experienced groups. It could also reflect increased numbers of SSPP's per synapse, of the same size. While we cannot rule this out, there was no statistically significant difference in the frequency of synapses with more than one SSPP across groups ($\chi^2 = .23$; $P > .25$). Finally there is the possibility that some proportion of apparent SSPP's actually are formed by irregularities in the edge of the subsynaptic plate. This can only be determined from serial sections and graphic reconstruction of the plate. Peters and Kaiserman-Abramof (*3*) presented reconstructions of 55 subsynaptic plates. Of these, 34 had SSPP's. To assess relationships between their plates and transverse sections, we superimposed potential planes of transverse section at 0.05-μm equivalent intervals and repeated the process at four successive orientations separated by 45°, yielding a total of 2330 simulated planes of section. Only 21 of these intersected an edge irregularity such that it would be interpreted as an SSPP. Since there is no reason to assume that our cortical synapses are different from theirs, we would expect only about six edge irregularities to have been misinterpreted as perforations in our total of 665 SSPP's. This analysis also indicated that the probability that a section through a plate revealed an SSPP, given that one or more were present, was about .45. This suggests that the "true" frequencies of SSPP's might be obtained by multiplying the figures in this report by the reciprocal, 2.2.
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Hippocampal Aging and Adrenocorticoids: Quantitative Correlations

Abstract. *Altered neural-endocrine relations have been proposed as factors in mammalian aging. In the same rats from three age groups we quantified astrocyte reactivity in hippocampus, performed radioimmunoassays for plasma adrenocorticoids, and measured adrenal weight. These variables were correlated in individual animals and generally increased with age. The findings are consistent with recent hypotheses that endocrine levels are related to brain aging, either as cause or effect.*

Recent neurochemical (*1*) and neurophysiological (*2*) findings suggest that deficits in synaptic transmission processes occur in the brain during aging. Combined with other data (*3*), some of these findings have led to the hypothesis that alteration of neuroendocrine control mechanisms may be an important pacemaker of the mammalian aging process; that is, initial age-related alterations in brain synaptic function could lead to gradual changes in neural control of endocrine processes, which, in turn, could lead to "cascading" physiological imbalances and age-correlated physical deterioration (*4*).

However, this view does not deal directly with the etiology of the initial brain deterioration. In this latter context, it has recently been proposed that hormones, even within normal ranges, may partially contribute to the initial deterioration of their brain target cells through gradual and prolonged catabolic actions (*5*). Thus, a "runaway" positive feedback loop between brain and endocrine phenomena, according to these views, could participate in the mammalian aging process.

Wexler and associates (*6*), in particular, and others (*7, 8*) have shown that elevated adrenocorticoids induce pathological somatic syndromes that are highly similar in pattern to the syndrome accompanying mammalian aging. Moreover, there is evidence of spontaneously elevated resting plasma adrenocorticoids in aging breeder or virgin male rats (*6, 9*), and adrenocorticotropic hormone (ACTH) release may be less suppressible in aging rats (*9*). However, some investigators have found an apparent reduction of maximal adrenocortical function during aging (*10*).

Given the similarity of the hyperadrenocorticoid and aging somatic syndromes, the adrenal system appears to be appropriate for examining the possibility that neural and endocrine changes are somehow related during aging (either as cause or effect). There have been, to date, no tests of the possibility that brain and endocrine alterations during aging are quantitatively correlated, but this

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possibility is seemingly predicted by either of the recent brain-endocrine interactive hypotheses of aging (*4*, *5*).

The major target cells of glucocorticoids in the brain have been found by McEwen and associates to be hippocampal and other limbic neurons (*11*). Moreover, glucocorticoids and ACTH influence hippocampal electrical activity and brain neurochemistry (*12*). Conversely, numerous investigators have reported that electrical stimulation or lesions of the hippocampus or its efferents can either increase or decrease ACTH release (*13*, *14*). These reciprocal interactions seem of interest with regard to aging because of the well-established early deterioration observed in the hippocampus in senile dementia, Alzheimer's disease, or even during normal aging (*15*).

We previously found that a quantitative, progressive, and reliable increase in reactive astrocytes (hypertrophied soma, thickened processes) occurs in the hippocampus (and, to lesser degrees, in caudate nucleus and other brain structures) of aging Fischer rats. This reactive glial increase begins at least halfway through the life-span, and has been quantified in light microscopic studies (*16*) and ultrastructural studies (*5*). (The latter finding seems to rule out differential staining as a basis of the light microscopic aging effect.)

In the present studies, we employed this early onset, quantitative measure of age-related hippocampal pathology to test the prediction of a correlative relation between brain and endocrine alterations during aging. In animals of three different age groups we quantified brain glial changes and assessed plasma concentrations of adrenocorticoids by radioimmunoassay. Adrenal weights were also measured. Our data indicate the presence of a quantitative relation between hippocampal pathology and adrenal activity during aging, and therefore seem to provide the first direct evidence of a correlative link between brain-endocrine functions in the aging process. Some of these data have been reported in preliminary form (*5*).

Our subjects consisted of nine young (4 months old), nine mid-aged (13 months old), and nine aged (25 months old) male inbred virgin Fischer 344 rats. The animals were obtained from a pathogen-free colony (*17*). The rack of animals was moved from the animal housing to the laboratory at 9:00 a.m., and the animals were killed by cervical dislocation and decapitation, beginning at 12:30 p.m.

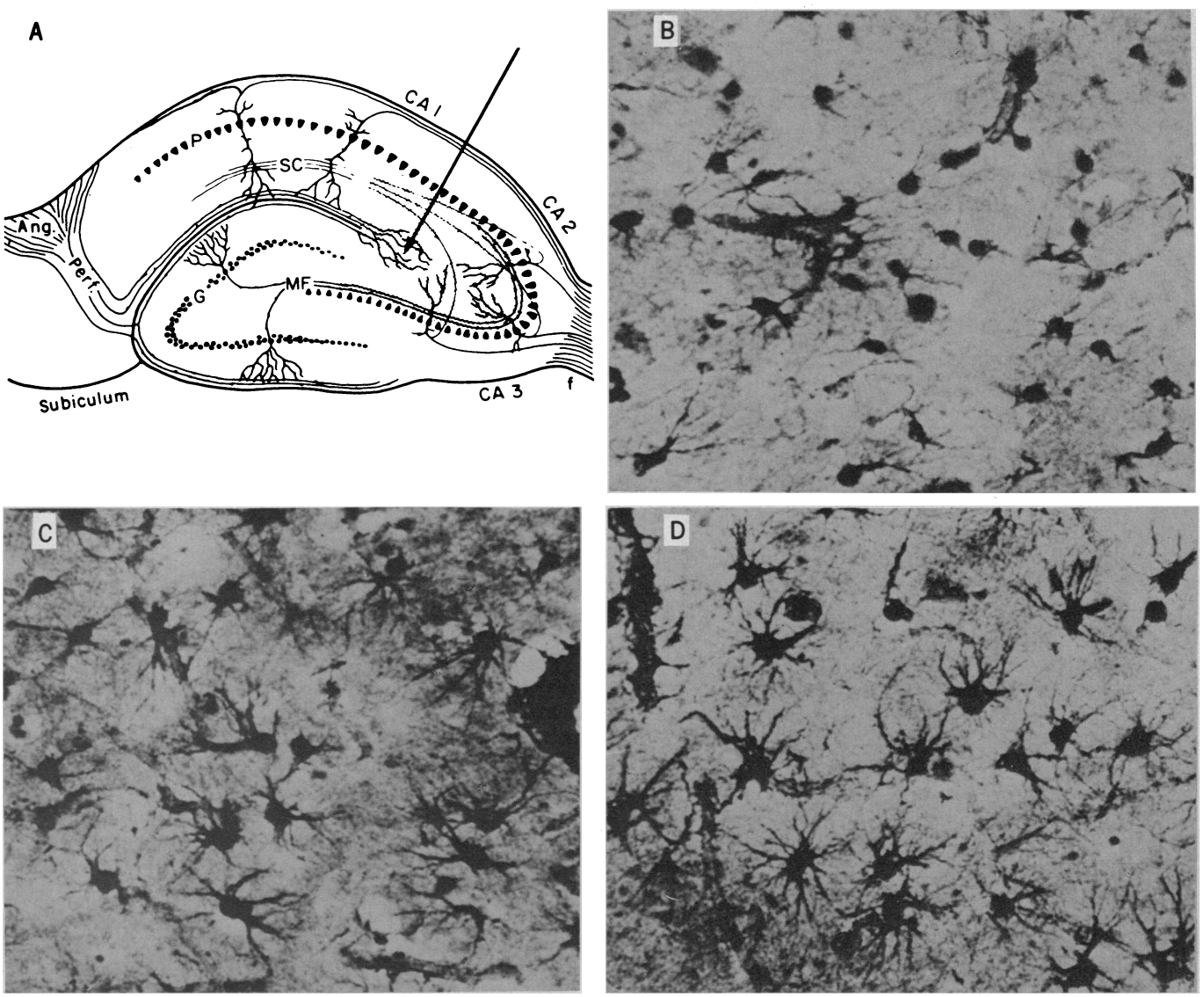

Fig. 1. (A) Schematic representation of a coronal slice from hippocampus of a rodent, modified after Golgi drawings of Cajal and Lorente de No' (*23*). The stippled region shows the area quantified in this study. (B) Astrocytes from the stratum moleculare-lacunosum of CA 3, where the perforant path fibers terminate, of a 4-month-old rat. (C) Astrocytes from the same region of a 13-month-old rat. (D) Astrocytes from the same region in a 25-month-old rat. The quantified region consists primarily of apical dendrites of pyramidal cells from CA 1, CA 2, and CA 3 and several major fiber paths. Cells in the dendrites are comprised primarily of astroglia, with some neurons, microglia, and oligodendroglia (*23*).

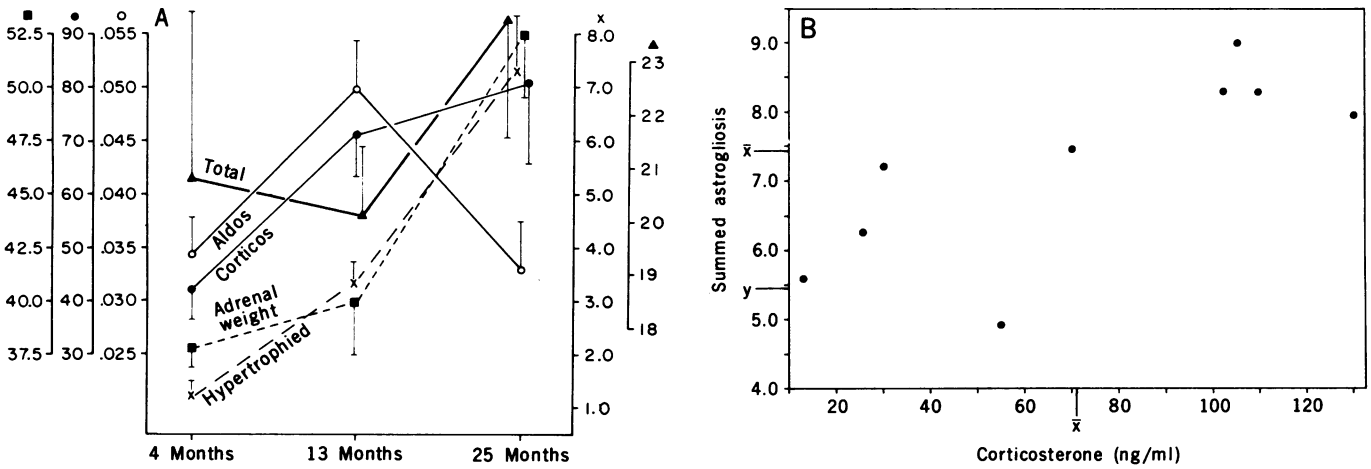

Fig. 2. (A) Mean values of plasma adrenocorticoids (nanograms per milliliter), adrenal weights (milligrams per pair), density of hypertrophied (reactive) astrocytes per grid square, and total astrocytic cells per grid square within the quantified region, at three different ages. (B) Correlation between concentrations of plasma corticosterone and summed hippocampal gliosis, a measure incorporating both total and reactive astrocytes (*19*). Plasma of each animal was analyzed for aldosterone and corticosterone with the use of radioimmunoassay kits prepared by commercial suppliers and according to their standard instructions. The aldosterone kit was obtained from New England Nuclear, and the corticosterone kit was prepared specifically for our studies (Radioassay Systems Laboratories, Carson, Calif.) (*18*).

Each animal was killed (within 1 minute of first being handled) in a part of the laboratory well removed from the remaining animals. Trunk blood was collected through a funnel into a heparinized test tube packed in ice. The tubes were centrifuged at 1500*g* for 15 minutes, and plasma was collected and frozen at −20°C until analyzed by radioimmunoassay (*18*). Both adrenal glands were dissected free, stripped of connective tissue and fat, and weighed as a pair. Brain sections (25 μm thick) were stained with Cajal's gold method and analyzed quantitatively for astrocytes (*19*). This method clearly demonstrates reactive astrocytes in brains of aged rats (*16*).

We focused our correlative analysis on the mid-aged group in order to determine whether any endocrine alterations might be associated with the etiology of changes in the brain due to age. As noted, hippocampal glial reactivity is readily detected as early as mid-life in Fischer rats. Such correlational analyses were not well suited for the other age groups because of the minimal degree of glial reactivity in young animals and because of evidence that adrenal functions may first be elevated and then adrenal histology may deteriorate in aged rats. This might make concentrations of circulating corticoids in the aged group an unreliable indicator of the levels of adrenal activity that had obtained during most of the animals' life-spans (*6*, *9*) (also see below). Brain sections were therefore quantified from all nine mid-aged animals, and from six young and six aged animals chosen at random.

Figure 1, B, C, and D, show representative examples of astrocytes from the region of perforant path terminals [a highly reactive region (*16*)] at the three different ages.

Figure 2A shows that a progressive and highly significant (*20*) ($P < .0005$) increase in mean density of reactive astrocytes occurs in hippocampus with age. Post hoc comparisons showed that the 13-month-old animals were different from 4-month-old animals ($P < .05$), while animals 25 months old also were different from those 13 months old ($P < .01$). Total glial cell density does not significantly increase with age, because of variability, although a nonsignificant mean increase of approximately 20 percent is observed in the aged group (*19*). The adrenal system in these animals also undergoes change as a function of age; adrenal weight exhibits a highly significant increase with age (*20*) ($P < .0005$). Adrenal weight is increased nonsignificantly in the mid-aged animals, and then increased substantially in animals from 13 to 25 months old ($P < .01$; Keuls-Tukey).

Aldosterone and corticosterone are significantly increased in the mid-aged as compared to the young group ($P < .05$) (*20*). In aged animals concentrations of these corticoids again tend down toward those of the young animals. Although the mean concentration of corticosterone seems increased in aged animals, this is an inflated measure due to two extremely elevated values in that group (180 to 270 ng/ml) which were at least 50 ng/ml above any other values measured in this study. Median corticosterone values in the aged animals are similar to those in the young rats. Thus, an apparent pattern of initial (mid-aged) increase, followed by a possible subsequent decline in adrenocortical function, is suggested. Similar patterns have been noted by others (*6*, *9*, *10*). This could partly account for conflicting data in the literature on plasma corticosterone during aging, since much might depend upon the relative physiological ages of subjects in the various studies.

Figure 2B shows the degree of correlation in mid-aged animals between a measure of summed gliosis [which gives approximately equal weight to total glial cells and to reactive cells (*19*)] and plasma corticosterone. Corticosterone concentrations were significantly correlated with astrocyte reactivity (with summed gliosis, $r = +.76$; with reactive cells alone, $r = +.65$; $P < .05$). The corticosterone correlation with total cell density was not quite significant ($r = +.45$). Plasma aldosterone in mid-aged animals exhibited lesser, nonsignificant positive correlations with reactive cells ($r = +.49$) and with summed gliosis ($r = +.38$). For reasons noted above, such correlations were not expected in the other two age groups. Nevertheless, the possibility that these relations may exist as early as 4 months is suggested by a similar correlative trend between reactive astrocytes and plasma corticosterone ($r = +.55$) in the six young animals we analyzed. Additionally, in the six aged animals in which glial cells were quantified, the correlation of adrenal weights with reactive cells was +.72 ($P < .05$), and with summed gliosis the correlation was +.65. This latter finding is again consistent with the possibility that brain astroglial changes are somehow related to levels of adrenocortical activity. Since there is good evidence of major deterioration of adrenocortical histology (and probably of secre-

tory functions) in aging rats (*5*, *6*, *9*), adrenal weight may be a better indicant in the aged animals of the past levels of (ACTH?) stimulation of adrenal gland activity and corticoids (that is, those which obtained during most of the animal's mature lifetime) than are present concentrations of circulating corticoids.

These correlations between brain glial reactivity and adrenal function, both in terms of age of onset and among individual subjects, provide evidence that endocrine functions and brain aging may in some way be associated. The data are consistent with both the neuroendocrine alteration hypothesis (*4*) and a recently proposed model which suggests that endocrine systems, in particular the adrenal steroids, can directly induce brain changes of the type seen in aging (*5*). Of course, any correlation may be a consequence of some third factor (for example, general brain or somatic deterioration) affecting both variables independently. Nevertheless, in view of the observed relations, it would appear important to test the prediction of the latter hypothesis that prolonged manipulation of peripheral hormonal systems should produce changes in hippocampal measures of astrogliosis; that is, of a pathological process that seems to be an early and sensitive index of brain aging. It is interesting that some morphological changes have been reported to occur in brain following prolonged administration of high doses of glucocorticoids (*21*), although no studies have yet been conducted on glucocorticoid administration and specific brain correlates of aging.

Note added in proof: Since submission of this report we have completed a 7½-month study in which ACTH, gluco- and mineral corticoids were administered in moderate doses three times weekly to aging rats. Astrocyte reactivity was elevated significantly in animals with elevated glucocorticoid activity. Also, the correlation of brain pathology and adrenal weight in aged controls, as reported here, was replicated. A preliminary report is in press (*22*).

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17. The barrier-reared, largely pathogen-free colony is maintained at Charles River Co. under contract to the National Institute on Aging and supervised by Dr. D. C. Gibson of the NIA. Because of the expense of raising these animals under pathogen-free conditions, numerous parts of the animals were also used for separate experiments not reported here. This necessitated killing the animals over a period of approximately 5 to 6 hours because of the dissection procedures. However, diurnal effects on hormone levels were controlled for by killing the animals in triplets (young, mid-aged, aged) and by employing statistical comparisons for paired samples (*20*). Additionally we investigated the contribution of time of killing to plasma corticosterone by performing rank-order correlational analyses between these two variables. The rank correlation values were, in aged animals, $r_s = -.08$ in mid-aged animals, $r_s = +.08$; and in young animals, $r_s = -.38$. The latter value, though nonsignificant, suggests that if order of killing affected corticosterone values, it did so only in the young animals. This correlation also shows an opposite sign from that expected if an afternoon diurnal rise in hormone levels had greatly affected the range of values. These observations suggest that basic individual differences, rather than diurnal factors, contributed the greatest part of the variance in corticosterone concentrations found among individual animals. All animals were killed prior to 1 hour before the onset of their normal dark cycle at 1900.
18. Radioassay Systems analyzed corticosterone concentrations of our rat plasma samples and their values were within a few nanograms per milliliter of our own for each sample they studied (the samples spanned our full range of values). Despite major differences in immunoassay procedures for the two steroids (because of very low plasma aldosterone values in rats), there was a highly significant correlation between aldosterone and corticosterone in individuals across all age groups ($r = +.7$; $P < .001$) which, according to the recent literature showing concomitant variation of aldosterone with corticosterone [see C. Gomez-Sanchez, O. B. Holland, J. R. Higgins, D. C. Kem, N. M. Kaplan, *Endocrinology* **99**, 567 (1976)], would be expected.
19. The brain was removed quickly after decapitation, postfixed in 10 percent formalin and then in a solution of formalin ammonium bromide (FAB), in preparation for staining by a modification of Cajal's gold sublimate stain (*16*). Brain tissue was sectioned coronally at 25-μm intervals, and every third section through the hippocampus was stained. One mid-polar hippocampal section was randomly selected for quantification from each animal without knowledge of the hormonal data. A grid was superimposed over the quantified area, using a drawing tube, camera lucida method. Total glial cells and clearly reactive cells were counted in each square. The rank-order correlation of reactive glial counts in mid-aged animals between two independent observers was +.81, and significant correlations between glial counts and corticosterone in mid-aged animals were found by both observers. We found nearly significant tendencies both for total cells, uncorrected for structure size, and for depth of the hippocampus to be increased in older animals. Diamond, Johnson, and Ingham [*Behav. Biol.* **14**, 163 (1975)] also found increased hippocampal size with age. Thus, decreasing structure size does not account for increased reactive cell density with age. Because of the tendency ($P < .1$) of total cells uncorrected for structure size to increase with age, and because of nearly significant correlations between total glial cell density and plasma corticoids (see text), we incorporated total cells with reactive cells into a measure termed "summed gliosis." Reactive cells in mid-aged animals accounted for approximately 15 percent of total cells; we therefore multiplied each animal's reactive cell density value by 0.15, and summed the result with that same animal's reactive cell density value. This provided a measure (summed gliosis) which gave approximately equal weight to reactive and to total cell densities. However, results are also significant when reactive cell density alone is used (see text).
20. Statistical comparisons of age group mean differences in adrenal weights and cell density counts utilized analyses of variance. Post hoc comparisons of differences between any two of the three groups employed the Keuls modification of Tukey's methods. However, plasma hormones, in particular corticosterone, could not be analyzed parametrically since Cochran's test showed that the assumption of homogeneity of variance was rejected at $P < .01$ (for example, Fig. 2A). Nonnormality of the hormonal distributions was also apparent. Some of this variability may have been contributed by killing the animals over a period of several hours (*17*). In order to partially compensate for this temporal vari-

ance component, statistical tests for paired comparisons were used, in which pairs of animals killed in the same triplets were matched when comparing two groups. Both nonparametric (signed-rank test, sign test) and parametric (*t*-test) paired comparisons showed an elevation of corticoids in the mid-aged groups over the young group ($P < .05$, two-tailed). Only one young animal exhibited higher corticoid values than its mid-aged paired animal. The aged group, however, was not found to be different in concentrations of corticoid from either of the other groups because of the extreme variance and bimodal distributions found in aged animals. Elevations in plasma corticosterone of rats with age have also been reported by three other research groups (*6*, *9*) [see B. J. Winer, *Statistical Principles in Experimental Design* (McGraw-Hill, New York, 1962)].

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Memory Impairment in Epileptic Patients: Selective Effects of Phenobarbital Concentration

Abstract. *Nineteen epileptic patients were tested first under medium (week 1) and then under high (week 2) therapeutic levels of phenobarbital. Relative to response times of 20 controls with equivalent practice but without medication, response times of patients in a short-term memory scanning task were strikingly slowed during week 2. However, increased phenobarbital did not slow responses in a task requiring access to information in long-term memory.*

In patients with recurrent epileptic seizures, the optimal daily dosage of anticonvulsant necessarily must represent a compromise between increased control of seizures and reduced information-processing capability. Although there are satisfactory ways of assessing both the patient's anticonvulsant level and his or her seizure frequency (*1*), no adequate measures have been advanced for evaluating drug-related cognitive impairment in epileptic patients (*2*). The present study was designed to assess the effect of one commonly prescribed anticonvulsant (phenobarbital) on specific parts of the memory system.

Cognitive theories distinguish two information stores in memory, a temporary short-term store and a more permanent long-term store (*3*). Much literature supports this dichotomy at the behavioral level (*4*) and, to a lesser extent, at the physiological level (*5*). Accordingly, we selected two tasks, each of which examines access to simple, highly familiar information in one of the memory stores. We were guided in our choice of tasks by previous investigations in our laboratory of the relation between verbal ability and memory (*6*).

Our measure of short-term memory performance was the Sternberg scanning task (*7*). In this procedure, a series of from one to six different digits is presented sequentially, followed after a brief pause by a probe digit (for example, 6 3 4 7 . . . 3?). The subject's task is to indicate as rapidly as possible whether the probe digit was in the memory set. The dependent variable is the reaction time (RT) required for the response, accuracy being perfect. Reaction time is typically found to be a linearly increasing function of the number of digits scanned.

There are several theoretical interpretations of this finding. The most straightforward is that the items are scanned one at a time in sequence. Under this interpretation, the slope of the linear function is taken as a measure of speed of access to information being held in short-term memory. Of course, other explanations are also possible. For example, one can develop models in which the search process takes place in parallel, and still account for the linear function (*8*). Other theorists have proposed memory strength models. However, for the purposes of this report, such distinctions are irrelevant. All the various models of the short-term memory scanning task assume that the slope of the linear function measures efficiency of access to information in short-term memory. That is all that we require. Slope values typically vary from 30 to 40 msec in normal, well-practiced college students.

As our measure of long-term memory performance, we used Posner's letter-matching task (*9*), a paradigm for investigating automatic access to name codes. On each trial, two letters are presented simultaneously and the subject judges whether the two letters have the same name. Thus, the correct response to "AB" or "Ab" is "different," while the correct response to "AA" or "Aa" is "same." Again, performance is essentially error-free.

This task can be used to measure the speed of access to long-term memory. Let us consider the simplest model of the task. It assumes that physically identical items (for example, "AA") can be responded to without the name even being determined; because the visual patterns are identical, the pattern names must be the same. On such trials, access to long-term memory does not involve the letter names. By contrast, detecting that "a" and "A" have the same name requires that the name codes of each pattern be retrieved from long-term memory and compared. Indeed, the RT on "name identity" trials is typically about 80 msec longer than the RT on "physical identity" trials when college students are used as subjects.

As is the case in the short-term memory scanning task, alternative models of the letter-matching task are also possible. For instance, the "horse race" (*10*) model assumes that visual patterns are always processed to a name level. In this model, the difference between name identity and physical identity trials is due to the extra processing required to deal with the entry of two nonidentical patterns into long-term memory. Once again, for the purposes of this report, the precise model for the task can be disregarded. All we claim for our task is that it is a measure of speed of access to information in long-term memory.

The epileptic patients performed the two tasks during week 1 under a medium maintenance level of medication (8 to 15 μg of phenobarbital per milliliter of blood) and during week 2 under a higher level (20 to 32 μg/ml). These dosages are representative of the levels used clinically. Approximately 30 percent of all epileptic patients are prescribed maintenance dosages at our lower level and 40 percent at our higher level. The medium dosage consistently preceded the high dosage because of the long half-life of phenobarbital, the hospital schedules, and the time which the patients had available. There were 3 days of testing on these tasks at each level of medication, separated by 5 days to permit the increased level of phenobarbital to stabilize. Day 1 of each week was considered to be a practice day, and the data were discarded. On days 2 and 3, several practice trials preceded each task to help reduce warm-up effects.

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