the red algae *Cystoclonium purpurem* of a peroxidase that can use both bromide and chloride as halogenation substrates. Peroxidases capable of oxidizing chloride could give rise to chloromethanes by a mechanism similar to the bromoperoxidase reaction. Peroxidative halogenation with either $I^-$, $Br^-$, or $Cl^-$ is therefore responsible not only for the synthesis of a wide variety of marine halometabolites but possibly also for contributing large quantities (*16*) of the more volatile halogenated hydrocarbons to ocean waters and the environment. In light of recent environmental concern over halohydrocarbon-catalyzed destruction of ozone (*19*), it would be important to measure the production of halocarbons by marine organisms and compare it with industrial halocarbon production.

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# Subsynaptic Plate Perforations: Changes with Age and Experience in the Rat

Abstract. *The relative frequency of appearance of discontinuities in the postsynaptic thickening, or perforations in the subsynaptic plate, increased with age and experience. Rats reared from weaning in complex or social environments had a significantly higher proportion of occipital cortical synapses with perforations than did rats reared in isolation. In addition, the relative frequency of these perforations more than tripled between 10 and 60 days of age. Shifts in the frequency of perforations can occur independently of changes in the size of synapses. This result suggests a new potential mechanism of synaptic plasticity.*

Synapses in certain layers of the occipital cortex have longer postsynaptic thickenings in rats reared under environmental complexity (EC) than those reared under impoverished conditions in normal laboratory cages (IC) (*1*, *2*). The thickenings were measured because they reflected the area of the synaptic contact and thus, possibly, synaptic efficacy. In these studies, we measured along the length of the thickenings, ignoring the gaps which sometimes appeared in them, as seen in Fig. 1.

Peters and Kaiserman-Abramof (*3*) used serial sections to demonstrate that, in the third dimension, these gaps in the postsynaptic thickening corresponded to irregularly shaped perforations in a subsynaptic plate; they also found that these subsynaptic plate perforations (SSPP's) were more frequent in larger plates. We were intrigued by their suggestion that the edge of the subsynaptic plate might be the active site of the synapse and, thus, that perforations in the plate might functionally strengthen the synapse. Given this different possible anatomical measure of synaptic efficacy, we decided to analyze the percentage of synapses in which these SSPP's appeared in our EC and IC rats (*1*). In reanalyzing data from these animals, in new data from socially housed (SC) animals, and in a new group of EC-SC-IC triplet sets, we have noted that the percentage of occipital cortex synapses in which SSPP's appear is affected by the postweaning environment of the animal and that the frequency of SSPP's may vary independently of the size of the synapse. In addition, the frequency of SSPP's increased with age in a third study. To our knowledge, the results reported here are the first experimental evidence that SSPP's might have a functional role.

The initial observation involved four littermate pairs of male Long Evans hooded rats in which postsynaptic thickening differences were previously described (*1*). One member of each pair had been reared under EC and the other under IC. We compared the rate of occurrence of SSPP's of the type shown in Fig. 1 in round vesicle, asymmetric synapses (*4*) of layer 4 of the occipital cortex, in which the greatest and most consistent difference in the length of the postsynaptic thickening occurs (*1*, *2*). In this layer, an unweighted mean of 18.8 percent [standard error of the mean (S.E.M.) = 1.4 percent] of these synapses in EC animals contained SSPP's visible in transverse section, whereas only 11.9 percent (S.E.M. = 2.4 percent) of these synapses in the IC animals contained SSPP's. Each of the four EC rats had a higher percentage than its IC littermate [$F$ (1, 6) = 5.96; $P \leq .05$].

Table 1. Total number of synapses and percentages in which SSPP's appear in postsynaptic thickening. Also included are comparisons of pairs of rats, which were reared for 30 days under the treatment conditions.

| Layer | Treatment group | | | | | | Comparison | |
|---|---|---|---|---|---|---|---|---|
| | EC | | SC | | IC | | | |
| | Synapses (No.) | Percent | Synapses (No.) | Percent | Synapses (No.) | Percent | EC > IC | SC > IC |
| | | | | *All synapses* | | | | |
| 1 | 1941 | 11.40 | 1790 | 10.25 | 1806 | 8.63 | 7/11 | 7/11 |
| 3 | | 14.84 | | 11.78 | | 9.69 | 7/11 | 8/11 |
| 4 | | 13.38 | | 13.59 | | 11.78 | 7/11 | 8/11 |
| | | | | *Large synapses* | | | | |
| 1 | 1007 | 22.27 | 913 | 21.78 | 941 | 14.80 | 8/11 | 8/11 |
| 3 | | 24.34 | | 22.97 | | 19.62 | 8/11 | 7/11 |
| 4 | | 22.50 | | 18.16 | | 16.08 | 7/11 | 8/11 |


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To study the reliability of this phenomenon, as well as the role of complexity as opposed to social condition in its generation, members of triplet sets of male Long Evans hooded rats were assigned at weaning (22 to 25 days of age) to one of three environments. One member of each triplet set was placed in EC, consisting of a group of 12 rats housed in a large cage filled with toys that were changed daily; in addition, they were allowed 30 minutes of daily exploration of a different toy-filled field. The second member of each triplet set was placed in IC, an individual standard laboratory cage. The third member was housed in a social cage (SC), a standard laboratory cage containing one other rat. After 30 days, the rats were assigned code numbers to prevent experimenter bias; after they were killed, blocks of occipital cortex were fixed and embedded (*5*). Transverse (coronal) sections 2 $\mu$m thick were taken from the tissue blocks, stained with *p*-phenylenediamine, and observed through a light microscope for determination of cortical layers. Adjacent ultrathin electron microscope sections were stained with lead citrate and uranyl acetate (*6*) and photographed with an electron microscope (JOEL JEM 100B) (final print magnification, ×41,800). Eleven of the 12 triplet sets (33 animals) were found to be adequately fixed and stained and were studied further.

Eight micrographs were made from each of layers 1, 3, and 4 of each subject, and all complete, transversely sectioned, round vesicle synapses with asymmetric postsynaptic thickenings were evaluated. The majority of these synapses appeared to occur on spines. Any break in the continuity of the postsynaptic opaque region of 0.05 $\mu$m or longer was counted as an SSPP. A total of 5537 synapses, an average of 56 per layer per subject, were studied (*7*).

Both EC and SC rats had an average of about 25 percent more synapses with SSPP's in all layers than did the IC rats, and approximately two-thirds of individual littermate paired comparisons favor the EC and SC rats (Table 1) (*8*). A 3 (environments) by 11 (litters) by 3 (layers within subjects) analysis of variance (*9*) indicated a significant effect of rearing environment [$F$ (2, 20) = 4.38; $P < .01$]. There were no other statistically significant effects or interactions. Planned comparisons showed the EC and SC means to differ from the IC mean ($F$ = 8.68 and 7.22; $P < .01$). There was no statistical significance between SC and EC treatments ($F$ = 1.54). Postsynaptic thickening length in all three layers paralleled other studies (*1*, *2*).

Table 2. Postsynaptic thickenings in layer 3 of rats reared for 130 days under two treatment conditions.

| Treatment group | Postsynaptic thickenings | | |
|---|---|---|---|
| | Number | Length ($\mu$m) | With perforations (%) |
| SC | 225 | .33 ± .01 | 19.5 |
| IC | 253 | .33 ± .01 | 13.9 |

As also observed by others (*3*, *10*), 90 percent of the synapses with SSPP's (596 of 648) were larger synapses—those above the median postsynaptic thickening length (approximately 0.25 $\mu$m). When only the subpopulation of synapses larger than 0.25 $\mu$m is examined (Table 1), a more sizable difference of approximately 35 percent between EC or SC and IC rats emerges. Since all synapses counted were of the round vesicle asymmetric type (*4*), there were no differences in synaptic morphology other than size that appeared to be associated with SSPP's.

The stimulation provided by a social environment during rearing appears from these data to be sufficient for the differential development of SSPP's. Further evidence of this, and also evidence that the phenomenon does not disappear after early development, is provided by experiment 2, in which members of five male littermate Long Evans hooded rat pairs were placed in either SC or IC environments for a period of 130 days beginning at weaning. Occipital cortical tissue was prepared in the same manner except that horizontal sections were taken at cortical layer 3 as determined from transverse light microscopic sections (*7*). Micrographs (final magnification, ×35,000) were analyzed for the length of the postsynaptic thickenings and the percentage of synapses in which SSPP's appeared. From 33 to 82 asymmetric, round vesicle synapses were analyzed from each subject.

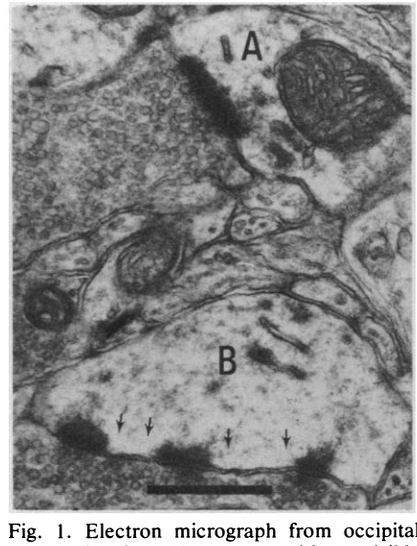


Fig. 1. Electron micrograph from occipital cortex showing (A) synapse without visible break in the postsynaptic thickening and (B) synapse with two SSPP's (arrows) in the postsynaptic thickening. (Scale bar, 0.5 $\mu$m).

There were no significant differences in the length of the postsynaptic thickening. (In only two pairs was the thickening longer in the SC rats than the IC rats.) However, the SC animals had 45 percent more synapses with SSPP's than did the IC animals [$F$ (1, 4) = 8.01; $P < .05$] (Table 2). Moreover, the difference was seen in each of the five pairs in this experiment, which may indicate a greater effect of a longer period of differential housing. This experiment indicates that the differential appearance of SSPP's need not be related to differences in length of the thickening or synapse size.

In preliminary research (*11*), one of us (T.J.D.) noted that the relative frequency of SSPP's increased in rats from a value of 3.6 percent of (168) occipital cortical synapses in a set of four males and four females at 10 days of age to a value of 12.4 percent (564 synapses) in a set of four male and four female littermates at 60 days of age; the postweaning period was spent in isolation ($\chi^2$ = 10.87; $P < .001$). There were no sex differences. Thus, the number of SSPP's increases with age as well as with differential experience.

Until now, two types of ultrastructural synaptic plasticity have been described following differential behavioral experience: changes in the size of the synapse or its structural components (*1*, *2*) and changes in the number of synaptic vesicles [for example, (*12*)]. These results add a third potential anatomical correlate of plastic change at the synaptic level: changes in perforations in the subsynaptic plate. This result is not merely a reflection of a change in the average size of synapses. Differences in SSPP frequency occurred independently of size differences in experiment 2.

The meaning of this difference in terms of synaptic function remains uncertain. The edges of SSPP's could, as Peters and Kaiserman-Abramof (*3*) suggested, be active sites. Other possibilities include (i) the nonstained region might contain a different postsynaptic material with reduced affinity for conventional stains or (ii) it might be devoid of normal subsynaptic proteins, perhaps to facilitate electrolyte or molecular movement across the membrane or to allow for up-

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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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-$\mu$m 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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