Local Differences in the Amount of Early Cell Death in Neocortex Predict Adult Local Specializations

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Local Differences in the Amount of Early Cell Death in Neocortex Predict Adult Local Specializations

Abstract. *The amount of early cell loss in five neocortical areas was inversely related to adult numbers of neurons in those areas. Differential cell death predicted particularly the thickness of the upper cortical laminae; it was not related to neuron numbers in the lower laminae. Cell loss thus determines some features of local neocortical differentiation.*

The mammalian neocortex is a structure of fundamental homogeneity on which local variations are imposed. These variations are of two types. The first is the number of cells in any unit volume perpendicular to the cortical surface. For example, a comparison of the number of neurons in a cortical column of defined width across six cortical areas in five species showed that, in monkey and man, the primary visual cortex had on average twice the number of cells per column as that in other cortical areas (*1*). In a study with conclusions supporting cortical homogeneity in number, the medial neocortex of rodents was excluded from the original analysis because of the obviously fewer number of cells in this area (*2*).

The second major variation in cortical areas is the relative number of cell types in the cortex—the several classes of pyramidal and stellate cells. Directly related is the particular type of afferent and efferent connectivity each cell type subserves. The differences can be major; for example, several of the subareas of cingulate and frontal cortex lack layer 4, while the primary visual and somatosensory areas have a highly developed layer 4. These local differences form the basis for the classical and much of the modern attempts to subdivide the neocortex (*3*, *4*).

In another neural structure, the vertebrate spinal cord, neurons along the length of the cord appear to be generated initially in roughly equal amounts and reach their eventual brachial, thoracic, and lumbar specializations by differential cell death (*5*). In order to see whether this same process might apply to the local differentiation of areas of the neocortex, we compared the amount and pattern of early cell degeneration in various cortical areas to their eventual cell number and pattern of laminar specialization.

We examined five areas of hamster posterior neocortex, defined by the criteria of Caviness and modified from Krieg (*3*): area 17, striate cortex; area 18b, parastriate cortex; areas 29d and 29b, cingulate cortices; and area 27 (also called 29a), variously termed cingulate, retrosplenial, retrohippocampal, or presubicular cortex (*4*). These areas include both the visual cortex and the thin medial cortical areas described by Bok (*2*) and exclude areas of extreme cortical concavity or convexity, where accurate estimation of numbers in any unit volume perpendicular to the cortical surface is difficult. The hamster is a particularly useful animal for this study, because the main period of neuronal death in the structures we examined occurs postnatally. Although cortical areas are not as differentiated in a hamster as in a gyrencephalic mammal, they are adequately differentiated, and the problems of compensation for gross cortical curvature can be avoided. A three to one range in cell number per unit column (Table 1), and a complete range of granular to agranular organization are found in these five areas.

To quantify adult cell numbers in the five cortical areas, a complete count of neurons in a column 200 μm wide, 30 μm deep, and height dependent on cortical area was made (magnification, ×500) from samples of both the right and left side of each of four hamsters. All tissue was processed identically: hamsters were killed with an overdose of urethane, perfused with 10 percent Formal-in-saline. The brains were embedded in albumin, frozen, cut in 30-μm coronal sections, mounted, and stained with cresyl echt violet. Coronal sections were chosen for counting in which the plane of section deviated less than 1 percent from perpendicular to the cortical surface. Samples were taken at points of minimal cortical curvature in the coronal plane, as measured by the ratio of the inner and outer perimeters of the cortex. For areas 27, 29b, and 18b, the perimeters differed by less than 5 percent, and no additional correction for curvature was necessary. For areas 17 and 29b, whose perimeters differed by 20 and 30 percent, respectively, at the points sampled, it was assumed that the external layers were thinned and the internal layers thickened by equal amounts by the curvature. Thus for these two cortical areas it was assumed that the total area in each lamina is conserved as it is stretched or compressed; the measured depth and the neuron number were thus modified accordingly (*2*). This method of correction compensates adequately for moderate amounts of curvature such as those described here; for larger convexities, cortical depth significantly increases. Finally, neuron counts were corrected for frequency of encounter by cell size by the method of Abercrombie (*6*).

Cortical areas in the newborn hamster were defined by reference to the areas in adult hamster. Coronal sections of the developing neocortex were anchored with respect to a variety of reference points, including the posterior extent of the caudate nucleus, the rhinal fissure; the hippocampal transitional zone, and the corpus callosum. The cortex was then subdivided with reference to the adult coordinates. These cortical areas are large with respect to the total amount of cortex present, and small errors in the placement of boundaries are unlikely to cause major errors in the accuracy of counts of entire areas. Postnatal days 5, 6, 7, 8, and 10 were examined, with up to three animals used per day per cortical area. These days were chosen because prior surveys revealed little cell death before day 5 or after day 10. By day 5, adult laminae are distinguishable, and migration of cortical neurons to the external laminae is terminating (*7*).

Table 1. Numbers of neurons in a unit column of various laminae of five cortical areas of the adult hamster and corresponding early cell death rates.

Cortical area	Adult neuron number					Summed degeneration rates		
	Upper layers		Lower layers		Total	Upper layers*	Lower layers†	Total‡
	2 and 3	4	5	6				
27	128 ± 21		142 ± 22		270 ± 25	66.8	6.2	40.0
29b	193 ± 29		133 ± 13	180 ± 25	505 ± 42	89.3	17.9	76.0
29d	253 ± 63	59 ± 35	198 ± 46	237 ± 89	747 ± 103	16.4	12.4	13.8
18b	202 ± 38	78 ± 23	130 ± 32	211 ± 38	595 ± 100	18.6	7.9	14.3
17	220 ± 40	114 ± 28	81 ± 18	245 ± 38	659 ± 78	14.1	6.6	12.1

*$r = -.85$. †$r = .38$. ‡$r = -.58$.

Animals were anesthetized and perfused with Formalin-alcohol, their brains embedded in paraffin, cut in 10-μm sections, and stained with cresyl echt violet for light microscopy. Three sections from each animal through the posterior neocortex were taken such that all five areas were included and counted for degenerating and normal cells. Degenerating cells were identified by a deeply stained and pycnotic nucleus with a liquefied appearance and pale or absent cytoplasm (*8*). Normal cells in the same areas were counted, and all degenerating cell counts are expressed as a ratio of the number of degenerating cells to the number of normal cells. These ratios were determined for each cortical area overall, and for layers within each area.

Degenerating cells can be found in the neocortex (Fig. 1 and Table 1), and the rates observed are comparable to those seen in other structures during development (*8*). The amount of degeneration is distributed quite differentially with respect to lamina (Table 1). Almost all the observed degenerating cells were found in external layers 2 and 3, and to a lesser extent in 4. Degeneration in 5 and 6 was small to negligible.

The amount of degeneration in the upper cortical laminae during development predicts their eventual thickness (Fig. 1). Rates of degeneration summed over days correlate negatively with the number of neurons in the lamina at maturity ($r = -.85$, $N = 5$, $P < .01$) (*9*). Although overall degeneration also correlates negatively with overall cortical cell numbers ($r = -.58$, $N = 5$, $P < .05$), the relation is due entirely to the effect of the upper laminae. In lamina 4, when one was differentiable, the amount of cell death did not predict laminar thickness; for example, area 17 has 1.5 times as many cells in lamina 4 as area 18b, but the sum of the degeneration rates is nearly identical (10.2 versus 10.6). Similarly, there was no evidence of a specific cell loss in a presumptive layer 4 that would serve to produce an agranular cortex in areas 27 and 29b. In the deep laminae, the small amount of cell loss was unrelated to the eventual number of cells in that lamina ($r = .38$, $N = 5$).

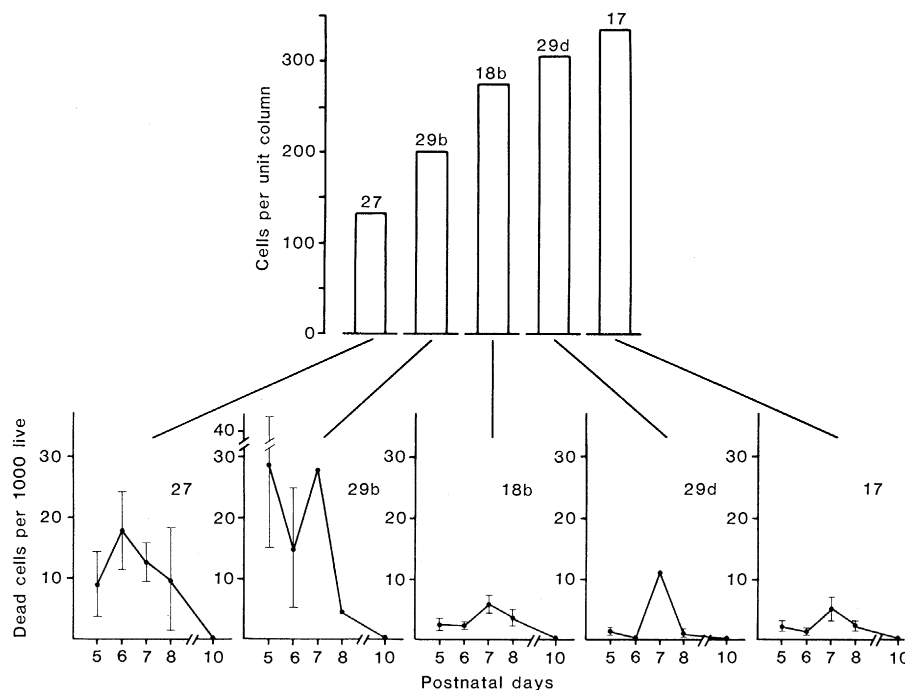

Fig. 1. Number of neurons in the upper cortical laminae in a column (200 by 30 μm with variable height) for five cortical areas and the corresponding cell death rates for days 5 through 10, where 1 is the day of birth. The upper cortical laminae include layers 2, 3, and 4 for 17, 18b, and 29d and layers 2 and 3 for agranular 29b and 27. Correlations of cell death rates and cortical numbers were calculated by summing the rates for the days shown to obtain a composite rate and then correlating this rate with adult cell number. High rates of early cell death in the upper laminae are associated with reduced cell numbers in those laminae at maturity.

Although the amount of cellular degeneration is related to end cell number, it should be emphasized that we have not quanitifed neuron loss directly. The rates of cell degeneration in the medial cortex were strikingly higher than those in any other neural structure, including the retina, tectum, thalamus, cerebellum, and basal ganglia (*6*). The rates in the upper laminae of 17, 18b, and 29d are comparable to those in other structures. For example, in the hamster retina, where the absolute amount of cell loss can be estimated, rates of degeneration correspond to a 50 percent loss of cells during development (*8, 10*).

Our results are consistent with reported cell loss during development in the telencephalon (*11*), and in the neocortex (*12*). In the study of mouse neocortex (*12*), a particular diminution of cell numbers in the upper cortical layers was also noted. We add that the early differences in the amount of cell degeneration can predict later differences in cortical thickness, particularly differences in number contributed by the upper cortical laminae.

Rockel *et al.* (*1*) suggested that cortical neurons are generated in an undifferentiated state and in a defined number that is the same for all cortical areas and that these neurons attain adult stellate or pyramidal morphology by virtue of the

afference to cortex in each zone. Our results are consistent with this interpretation, and we also suggest that cells are assigned to laminae as they arrive and from inside to out (*6, 13*) in direct proportion to the amount of afference, with any mismatch between the amount of cells generated and availability of afference (or efference) absorbed by cell death in the last generated, outside lamina.

Evidence from the reeler mutant mouse suggests that the connectivity and identity of cortical neurons is independent of laminar position (*14*), a finding that appears to be in conflict with our observations in the hamster. However, in reeler, cortical cells still occupy the same position relative to each other and are still stratified by birthday; only their position relative to the ventricular zone and the marginal zone is altered. It is possible that only the order of termination is altered and not the mechanism of cellular specification. In the reeler, we would thus predict that the last generated, inside lamina should show the most variability in cell loss.

Whether cell death plays a role in the organization of other cortical areas in other species as well as what controls a cortical cell's viability are not yet known. Studies of the loss of callosal and subcortical cortical projections in early development suggest that cell death is not involved in the error correction function of axon retraction (*15*). Rather, cell death appears to be a clue to the mechanism of specification of a cortical neuron's identity.

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Deficiency of a Kidney Metalloproteinase Activity in Inbred Mouse Strains

Abstract. *Kidneys from BALB/c mice contain a potent metalloendoproteinase, termed meprin, that is active against large proteins as well as small peptides. The enzyme is present in mouse strains C57BR/cdJ, C57BL/6J, BALB/cJ, A/J, DBA/1J, CD/1, Swiss, and ICR. Three related inbred strains, CBA/J, CBA/CaJ, and C3H/He, are markedly deficient in this enzymatic activity. This is the first report of a heritable deficiency of an intracellular proteinase in mammalian tissues. Meprin deficiency appears to have arisen as an early event in the development of the C stock. Furthermore, meprin is present in the progeny of a cross between a meprin-sufficient female (C57BL/6) and a meprin-deficient male (C3H/HeN), an indication that the trait for the deficiency is recessive.*

The study of heritable enzyme deficiencies in animals has contributed to our understanding of metabolic processes, and many specific deficiencies have been identified (*1*). Although many inherited hydrolase (E.C. 3.x.x.x) deficiencies have been reported, only a few inborn errors of metabolism involve one subdivision of this group, the proteinases (subclass peptidyl-peptide hydrolases, E.C. 3.4.x.x). Furthermore, the known inherited deficiencies in proteinases are limited to extracellular enzymes such as the complement component C_3 and a collagen-processing peptidase (*2*). As far as we know, no inherited deficiency of an integral cellular proteinase has yet been documented. We present here data demonstrating such a deficiency.

Meprin (metalloendopeptidase from renal tissue) was first discovered and purified from kidneys of the BALB/c mouse (*3*). It is a membrane-bound endopeptidase that degrades a wide range of substrates including large proteins. It is active optimally at alkaline *p*H values and is inhibited by compounds that typically inhibit metalloenzymes (EDTA,

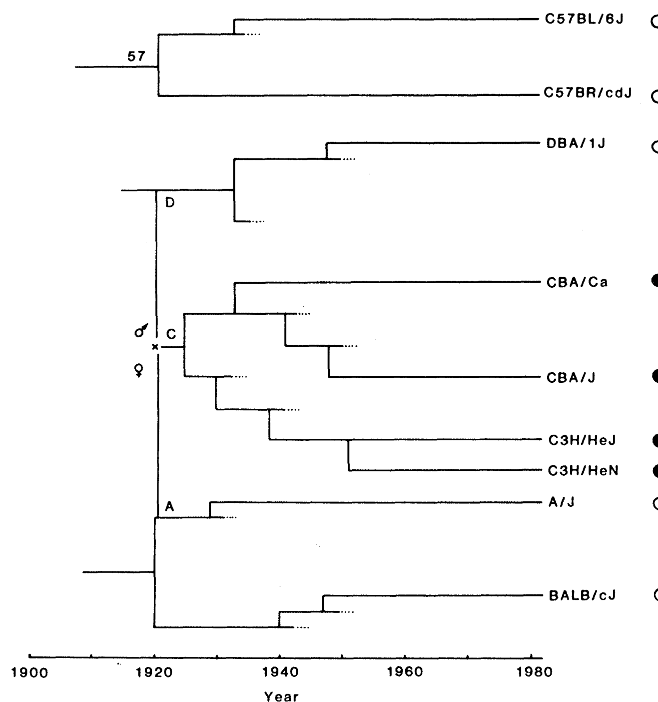

Fig. 1. The relationship between meprin deficiency and the origins of some inbred mouse strains. The genealogical chart was prepared from data in (*8, 9*). Strains that were classified as meprin-deficient are indicated on the right side by a closed circle; meprin-sufficient mice are indicated by an open circle. Ten years is approximately equal to 28 generations.