Neurons in Rhesus Monkey Visual Cortex: Systematic Relation between Time of Origin and Eventual Disposition

Abstract. Autoradiographic evidence after injection of tritiated thymidine indicates that cell position in the laminae of the monkey visual cortex is systematically related to time of cell orgin. The earliest-formed neurons, destined for the deepest stratum, arise at about embryonic day 45, and the last ones, destined for the outermost cell stratum, form at about day 102; cells of intervening layers are generated at intervening times. No neocortical neurons are produced in the last two prenatal months or after birth. Compared to cortical neurons in rodents, those in the monkey arise earlier, and the "inside-out" relation of cell position to time of origin is more rigid.

Morphological and physiological studies of the visual cortex in the monkey have increased our understanding of the organizational properties of the cerebral cortex in general (1). It is important to determine how such a complex and ordered system develops. Almost 100 years ago, it was demonstrated with the use of the Golgi method that cellular development of the mammalian cerebral cortex follows a consistent pattern, with large pyramidal neurons of the lower layers taking a laminar position and differentiating earlier than neurons destined to be situated more superficially (2). More recently, it was shown by autoradiography with [3H]thymidine ([3H]dT) that the deeper cortical neurons in rodents are generated earlier than the more superficial neurons (3). In addition, these studies provided direct evidence that cortical neurons originate in proliferative zones close to the ventricular surface and migrate to the cortical plate only after final division of the precursor cells.

It is essential to establish whether the neocortex in primates develops according to the same principle as in rodents and to determine the time span during which cortical neurons are generated in a slowly developing gyrencephalic brain similar to that of man. In addition, important questions concern the generation and migration rates of cortical neurons, the relation between laminar position and neuronal type with reference to time of cell genesis, and the possible interaction of young neurons with each other and with the specific afferents with which they will make synaptic contacts.

Several properties of the primary visual cortex (area 17) of the rhesus monkey make it a suitable model for study of these problems. The horizontal stratification of neurons into separate layers in this species is more precise than in most mammals, and area 17 can be distinguished from adjacent areas at relatively early embryonic stages. The large size of monkey fetuses allows adequate fixation for electron microscopy by vascular perfusion (4), and the protracted span of development increases the resolution of temporal sequences in neurogenesis (5). The present demonstration of the time of neuron origin for each of the six cortical layers is a



first step in the more detailed analysis of neocortical cell genesis in primates.

Ten pregnant monkeys weighing 6 to 10 kg were each injected once intravenously with [3H]dT (5 or 10 mc per kilogram of body weight) at 40, 45, 54, 62, 70, 80, 90, 102, 120, and 140 days of gestation. Females were caged with males from day 11 to 15 following onset of estrus, and the time of conception was estimated on the assumption that ovulation occurred on day 12. Pregnancy in the rhesus monkey lasts 165 days. All fetuses were delivered normally and killed 2 to 5 months after birth (the "juvenile" period, when most cortical cells have attained their final position). Two additional monkeys were injected 2 and 18 days after birth, respectively, and killed 3 months after birth. All brains were processed for autoradiography (5, 6). With the aid of a Zeiss microscope equipped with a drawing tube, the positions of heavily labeled neurons were recorded within a strip of visual cortex approximately 10 mm long situated in the depth of the calcarine fissure between the two arrows in Fig. 1A. Only heavily labeled cells are considered to have had their "birthday" on the day of [3H]dT injection (5, 6). [In the interpretation of autoradiographic data, the term "birthday" is empirically defined as the last day on which nuclear DNA is replicated in a given cell line (6).] The minimum number of grains for classification of cells as "heavily labeled" was arbitrarily determined for each specimen as half of the grain count neurons with maximum radioactivity. Brodman's nomenclature of layers was adopted (7).

No heavily labeled neurons were seen in the visual cortex of the 3-month-old monkey that had been exposed to [³H]-

Fig. 1. (A) Coronal section of the occipital lobe of a 3-month-old monkey. The two arrows indicate a strip of visual cortex about 10 mm long in the depth of the calcarine fissure, for which the time of neuron origin was analyzed in this study. The area in the rectangle is enlarged on the left side of Fig. 2. The 30-µm section was stained with cresyl violet $(\times 1.2)$. (B to H) Autoradiograms of the visual cortex in juvenile monkeys that had been injected with [3H]dT at various embryonic (E) days: E45 (B), E62 (C), E70 (D), E80 (E), E102 (F and G), and E120 (H). Roman numerals indicate cortical layers according to Brodman's classification (7). Arrows point to heavily labeled cells; M, solitary pyramid of Meynert; WM, white matter $(\times 650)$.

dT on embryonic day 40 (E40). However, a few lightly labeled neurons, located exclusively in the deepest part of the cortex, were found in more than 50 sections examined. Since heavily labeled cells were found elsewhere in the brain, these lightly labeled cells were probably the products of several cell divisions subsequent to the time of injection and it is unlikely that any visual cortical neurons were born at E40.

The first heavily labeled neurons were detected in the monkey injected at E45. They were localized in a narrow zone in the deeper portion of layer VI (Figs. 1B and 2A). Scattered neurons in the

white matter below layer VI were also labeled in this specimen. In most fields, a number of lightly labeled cells were detected superficial to the heavily labeled cells, an indication that cells generated later take up more external positions. This was confirmed by the finding that heavily labeled neurons in the animal injected at E54 were located somewhat more superficially, although still within layer VI (Fig. 2B). The majority of neurons generated at E62 were later situated in the upper twothirds of layer VI, while some were localized in layer V (Figs. 1C and 2C). Cells with axons passing from area 17



Fig. 2. Diagrammatic representation of the positions of heavily labeled neurons in the visual cortex of juvenile animals that had been injected with $[H^3]dT$ at various days of gestation (indicated at the top of each vertical line). On the left is a photomicrograph of a 30- μ m cresyl violet-stained section photographed at the same magnification used for plotting labeled neurons with the drawing tube. Horizontal markers on each vertical line except G indicate positions of all heavily labeled neurons encountered in a randomly selected strip (2.5 mm long) of the calcarine cortex. The three labeled neurons represented in G were found only after examination of 80 areas of calcarine cortex, each 2.5 mm long, in 40 autoradiograms of a single specimen. Roman numerals indicate cortical layers according to Brodman's classification (7); LV, obliterated posterior horn of the lateral ventricle.

to the midbrain, the so-called giant solitary pyramidal neurons of Meynert (8) which are situated in layers V and VI in the rhesus monkey (Fig. 1C), were also labeled by injection at E26.

At E70, the number of labeled neurons per unit length of visual cortex reached a maximum (Fig. 2). Injection at this stage labeled mainly neurons that later were located in layer V, and also many cells in layer IVC (Figs. 1D and 2D). Neurons generated at E80 became distributed over the entire width of layer IV, with the highest concentration in layer IVB (Figs. 1E and 2E); a few radioactive cells were situated in layer III. Injection at E90 labeled neurons in both layers III and II (Fig. 2F).

By E102, almost all neurons in the visual cortex had been born, since few neurons (less than 1 in 106) at the border between layer II and the cellsparse layer I were labeled in the 3month-old monkey that had been injected on E102 (Figs. 1F and 2G). However, in this specimen, some small nuclei situated mainly in the deeper half of the cortex were radioactive; these were classified as glia on the basis of small nuclear size, absence of Nissl substance, and satellite position with respect to neurons (Fig. 1G). This correlates with the time of appearance of numerous astrocytes in the depth of the fetal monkey visual cortex as assessed by the Golgi method (9). Injections at E120, later during gestation, or after birth did not label neurons of the visual cortex. However, numerous glial (Fig. 1H) and endothelial cells were labeled in the cortex in these specimens.

The neurons in the plexiform layer I were not labeled in any of the specimens studied. These neurons either were generated before E40, or they arose during a relatively brief interval between the ages sampled by injection of ["H]dT in this series of animals.

As shown in Fig. 2, the position of heavily labeled neurons in the juvenile monkey visual cortex is correlated with the time of cell origin in the fetus; cells destined for deep cortical positions are generated first, and more superficial ones at progressively later times. Thus, most of the neurons of layer VI are born between E40 and E60, neurons in layer V between E60 and E70, those in layer IV between E70 and E80, and those in layers III and II between E80 and E100. These data pertain only to the time of cell origin and do not define when the cells actually attained their final positions.

This study establishes that neurons generated on a day early in development eventually occupy a relatively narrow zone of the mature cortex, while those generated on a later day are eventually distributed over a somewhat wider zone; also, neurons born 8 to 10 days apart overlap somewhat in position along the radial axis (Fig. 2). One reason for the wide distribution of cells generated simultaneously may be differences in rates of migration. Slowermoving neurons might reach the cortex when it had increased several hundred micrometers in thickness by the addition of neurons that were generated later but had migrated faster. Within a population of simultaneously labeled cells, the slower-moving neurons would presumably take more superficial positions than the faster-moving ones. Indeed, initial examination of monkey embryos killed at shorter intervals after injection of [³H]dT suggests that some labeled neurons reach the outer border of the cortical plate in less than 3 days, whereas others labeled simultaneously take 7 or more days to reach a comparable destination (10). Variations in the length of the cell generation cycle also could influence eventual neuron position in the cortex, as might differences in the detailed aspects of cell interaction during migration (11).

Autoradiographic results in the brain of this primate corroborate in general the "inside-out" pattern of cell disposition described for rodents (3). Initial observations suggest that cells in other cortical areas in the monkey behave similarly, although on slightly different time schedules (10). Comparison of the data in Fig. 2 with studies in mice (12)shows that simultaneously generated neurons in the monkey eventually become confined to relatively narrow strata of the cortex; that is, the "insideout" principle is more rigidly followed in the monkey. This may be the developmental basis for the sharper boundaries of cortical layers in the visual cortex of adult primates.

The monkey neocortex acquires its full complement of neurons at relatively early stages of gestation. The last cortical neurons are generated close to birth in mice and rats, and cortical genesis continues for a few days after birth in the hamster (3). In contrast, the last neurons destined for the primary visual cortex are generated around E100 in the monkey, that is, about 2 months before birth. It is probable that other primates, including man, also acquire a full complement of neocortical neurons well before birth. PASKO RAKIC

Department of Neuropathology, Harvard Medical School, and Department of Neuroscience, Children's Hospital Medical Center, Boston, Massachusetts 02115

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Deuterium Oxide Effect on Temperature-Dependent Survival in Populations of Drosophila melanogaster

Abstract. A comparison of the mean life-spans for populations of Drosophila melanogaster at 10°, 20°, 25°, and 30°C maintained on media prepared with distilled water and with 50 percent deuterium oxide shows that deuteration decreases longevity at all four temperatures. The magnitude of the difference between the mean survival times of populations maintained on deuterated and nondeuterated media is inversely related to temperature between 10° and 30°C.

We have compared the survival of Drosophila melanogaster (Oregon-R) males maintained at 10°, 20°, 25°, and 30°C on medium (Carolina Instant Medium, Carolina Biological Supply) prepared with 50 percent D_2O with those maintained on medium prepared with distilled H₂O. The mean life-span of these Drosophila populations at



20°C was reduced from 85 to 29 days on a diet prepared with 50 percent D_2O . Furthermore, we found that reducing the temperature to 10°C caused a decrease in mean survival time from 29 to 7 days for populations maintained on the D_2O medium, whereas survival time was increased from 89 to 112 days for populations supplied with distilled H₂O medium. Raising the temperature from 20° to 25°C for populations supplied with D₂O medium increased survival from 29 to 36 days. At 30°C mean survival time dropped

Fig. 1. Mean survival time (\bar{S}) in populations of Drosophila melanogaster (Oregon-R) males maintained from 1 day of age on Carolina Instant Medium prepared with distilled H₂O or with 50 percent D₂O at temperatures (T) of 10° , 20° , 25° , and 30°C. The light-dark cycle was 12 hours-12 hours. Flies were reared at 20°C on standard corn meal-molasses agar. Ten tubes of 20 flies each were used for each determination of \overline{S} . (Inset) Difference between mean survival times for populations on D_2O medium [$\overline{S}(D_2O)$] and H_2O medium [$\overline{S}(H_2O)$].

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