Developmental Gradient of Cell Cycle in the Telencephalic Roof of the Fetal NMRI-Mouse

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Summary. An analysis of cellular kinetics in the NMRI-mouse after 12, 13, 15, and 17 days of gestation was obtained by means of tritiated thymidine autoradiography. After 12 and 13 days there is no significant difference in generation time between the lateral and the medial districts of the telencephalic roof. From 15 days of gestation onwards, the generation time in the lateral parts is significantly greater than in the medial wall regions. Simultaneously, on day 17 the growth fraction is drastically decreased in the lateral parts, while it remains close to 1 in the medial parts. Lengthening of the generation time during differentiation of the lateral wall is mainly due to an extension of the $G_1$-phase and to a lesser degree also of the S-phase. Another significant contribution comes from the increasing length of the mitotic phase.

Key words: Neocortical development – Cell cycle – Regional differences

Introduction

The existence of a gradient in the neurogenesis of the telencephalic roof has been described in rat and mouse (Hicks and D'Amato 1968; Smart 1973; Smart and Smart 1977) and has been found to comprise mainly a distinct lateromedial gradient. Similar findings have also been made regarding the diencephalon of the rat (Angevine 1970), the tectum opticum of the chick embryo (La Vail and Cowan 1971), and the spinal cord of the rat (Nornes and Das 1974). Although the cell cycle of the ventricular zone cells in rodents has been studied by many authors at different times of gestation (Atlas and Bond 1965; Kauffman 1968; Waechter and Jaensch 1972; Hoshino et al. 1973; Wilson 1973; Schultze et al. 1974; Shimada et al. 1977), the status of cellular proliferation and cell cycle with respect to the establishment of a developmental gradient requires re-investigation. Consequently, the present study was undertaken to provide a quantitative analysis of cellular kinetics for both the lateral and medial districts of the telence-
phalic roof in the normal mouse embryo on days 12, 13, 15, and 17 of gestation by means of tritiated thymidine autoradiography.

Material and Methods

Female virgin NMRI mice, 8 weeks of age, were mated between 8 a.m. and 10 a.m. and subsequently examined for vaginal plugs. The next morning was considered to be day 1 post conception (p.c.). Mother animals received a standard diet (Altromin) and water ad libitum and were housed in cages in air-conditioned animal rooms at 23°C with artificial light from 6 a.m. to 6 p.m. 120 pregnant females were given a single i.p. injection of 3H-thymidine (5 μCi/g; specific activity 5.0 Ci/mM) at approximately 9.00 a.m. either on day 12, 13, 15, or 17 of gestation.

Litters were removed at 1 h intervals up to 24 hours after injection. Two embryos of each litter were chosen at random for fixation in a mixture of equal parts 3% glutaraldehyde and 3% formaldehyde in phosphate-buffered solutions at pH 6.9. From this maximum number of 24 observations 15 were taken for investigation at 12 days of gestation, 14 at 13 days, 17 at 15 days, and 14 at 17 days of gestation. Each observation time is represented by 4 embryos selected at random from 2 litters. The embryos were embedded in paraffin, and cut in coronal sections of 5 μm thickness. The slides were dipped in Kodak NTB-2 emulsion (diluted 1:1 with water) at 55°C, and exposed in a light-opaque box at 4°C for 3.5 weeks. After this proper exposure period the autoradiographs were developed in Unifix at 38°C (7 min), fixed in sodium thiosulfate (8 min), and stained with hematoxylin and eosin. After mounting in Eukitt the autoradiographs of 15-20 consecutive sections of the middle telencephalic region were observed at a 400-fold magnification, whereby the deep section layer was excluded from evaluation, as far as possible. Nuclei containing 5 or more grains were considered labelled.

The percentage of labelled mitotic figures was separately registered for both the lateral and medial districts of the telencephalic roof at the level of the chiasma opticum. In default of another definite anatomical border, the distinction between lateral and medial districts was made in accordance with a vertical line at the level of the incisure between the lateral and medial ganglionic eminence. Although this does not exactly define the site of the measurement within the medial and lateral districts, the distinction allows comparison with other studies of developmental gradients of corticogenesis, such as the reports of Smart (1976) and Smart and Smart (1977). The ganglionic eminences are not included in our evaluation.

The mean percentages for 4 embryos were plotted graphically at intervals of 1 to 2 h. The range was less than 5% for each mean percentage.

In addition, mean mitotic indices (percentage of cells in mitosis) and mean labelling indices (percentage of ventricular cells labelled 1 h after 3H-thymidine application) were determined on a total of 1200 ventricular cells in each of 2 embryos which were taken after 1 h labelling time at each of the above mentioned 4 gestation stages. We further extended these observations on mitotic indices and labelling indices by injecting 3H-thymidine to an additional 6 pregnant mice on gestation days 14, 16, or 18. The litters were removed 1 h later and 4 embryos at each stage were processed for autoradiography. Evaluation was done separately for the lateral and medial hemispheric districts.

Calculations of the duration of each stage in the cell cycle were based on the method already described for cellular kinetic studies on pulse-labelled mice (Quastler and Sherman 1959; Kauffmann 1968; Schultze and Korr 1981). The total generation time read directly from the individual curves in Figs. 1 and 2 was equal to the interval between 2 identical points on 2 waves which most conveniently are represented by the 50%-points. The length of the S-phase (tg) was equal to the interval between the 50% points on the ascending and descending limbs of the curve. The length of the mitotic phase (tM) was calculated as the mitotic index/100 x generation time. The premitotic period (tg2) plus one-half the length of mitosis was determined as the 50% point on the ascending curve. The postmitotic period (tg1) was equal to the generation time minus the sum of tg, tM and tg2.

The total generation time (Tc) can also be calculated by the formula

\[ T_c = \frac{tg \times \text{labelling index}}{t_g} \]

which is equal to \( T_c = t_g \times \text{number of all cells in the cycle/number of S-phase cells} \)