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Cerebral malformation induced by prenatal X-irradiation: an autoradiographic and Golgi study

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INTRODUCTION

Exposure of the rat embryo to X-irradiation on the fourteenth day of gestation induces early cellular necrosis, partly removed by exogenous macrophages, and remodelling of the telencephalic mantle with the formation of ectopic germinal rosettes and development of an abnormal cortical pattern. This is characterised by a thin cerebral cortex, with its layering preserved, and large ectopic subcortical masses (Hicks, D'Amato & Lowe, 1959; D'Agostino & Brizee, 1966; Takeuchi, Shoji & Murakami, 1976; Ferrer & Sarmiento, 1983).

However, following irradiation with 150–200 rad on the sixteenth day of gestation, a bizarre cerebral cortex is produced, with abnormal lamination and neuronal nodules in its superficial half. Ectopic neuronal nodules in the hippocampus are seldom observed but subcortical ectopic masses are smaller than those observed at earlier ages. Prenatal irradiation on the eighteenth day of gestation produces neuronal cortical nodules in the superficial region of the cortex (Hicks *et al.* 1959; Hicks & D'Amato, 1966, 1978).

Similar abnormal developmental patterns have been reported in the mouse brain following prenatal X-irradiation at different stages of gestation (Dekaban, 1969; Schmahl, Weber & Kriegel, 1979).

From these earlier studies, it is known that ectopic rosettes are the germinal centres for subcortical masses, as are the lateral periventricular germinal centres for neuronal elements of the cerebral cortex. However, autoradiographic studies in which successive migratory waves of radioisotope-labelled primitive cells are followed to their definitive positions are necessary to elucidate the mechanisms of cellular migration. In addition, study of the fine structure of the abnormal telencephalic mantle, and more especially of the subcortical masses, is useful for a better understanding of the cellular remodelling occurring in these experimental models of cerebral malformation.

MATERIAL AND METHODS

Sprague–Dawley rats were irradiated with 200 rad in a single dose on the fourteenth, sixteenth or eighteenth day of gestation. The pregnant rats were additionally injected intraperitoneally with [^{3}H]thymidine, 6 μCi/g body weight, on either the sixteenth, seventeenth, eighteenth, nineteenth or twentieth day after conception. After delivery, the irradiated animals were killed under ether anaesthesia at different ages from newborn to 30 days old, and the brains fixed for histological, autoradiographic or cytological studies based on the rapid Golgi method (Ferrer & Martinez Matos, 1981).

For histological studies, the brains were fixed in 1 % glutaraldehyde containing 1 % formaldehyde in 0·12 M phosphate buffer, embedded in paraffin wax and the sections stained with haematoxylin and eosin or with cresyl violet as a Nissl stain.

For autoradiographic studies, dewaxed sections were immersed in Ilford K5 photographic emulsion, stored in dark boxes for one or two months and developed later with Microdol X. Sections were counterstained with cresyl violet. For studies based on the rapid Golgi method, the brains were fixed in 1 % osmium tetroxide containing 3 % potassium bichromate for four to 7 days and then immersed in 0·75% silver nitrate for 2 days. Sections were taken normal to the surface.

Normal (non-irradiated) animals were injected with tritiated thymidine and processed similarly for comparison with experimental animals. In addition, pregnant rats irradiated on the fourteenth gestational day were killed on the sixteenth or eighteenth day after conception and the fetuses removed by Caesarian section. The brains were fixed in 1 % glutaraldehyde containing 1 % formaldehyde in phosphate buffer for 24 hours. Small blocks of selected cortical regions were then post-fixed in Dalton's osmium tetroxide for two hours, dehydrated in ethanol and propylene oxide and embedded in Araldite. Semithin sections were stained by Richardson's method.

RESULTS

General morphological findings

Compared with the cortex in normal animals (Fig. 1A), rats irradiated on the fourteenth gestational day showed an atrophic cerebral cortex with a partially preserved lamination. No abnormal neurons were present in the molecular layer. Layers II–IV of the cortex were poorly defined and were formed by small and medium-sized pyramidal cells. Large pyramidal cells were the main cell type in layer V. Layer VI contained neurons of small size; horizontal neurons were identified in the deep part of this layer (Fig. 1B).

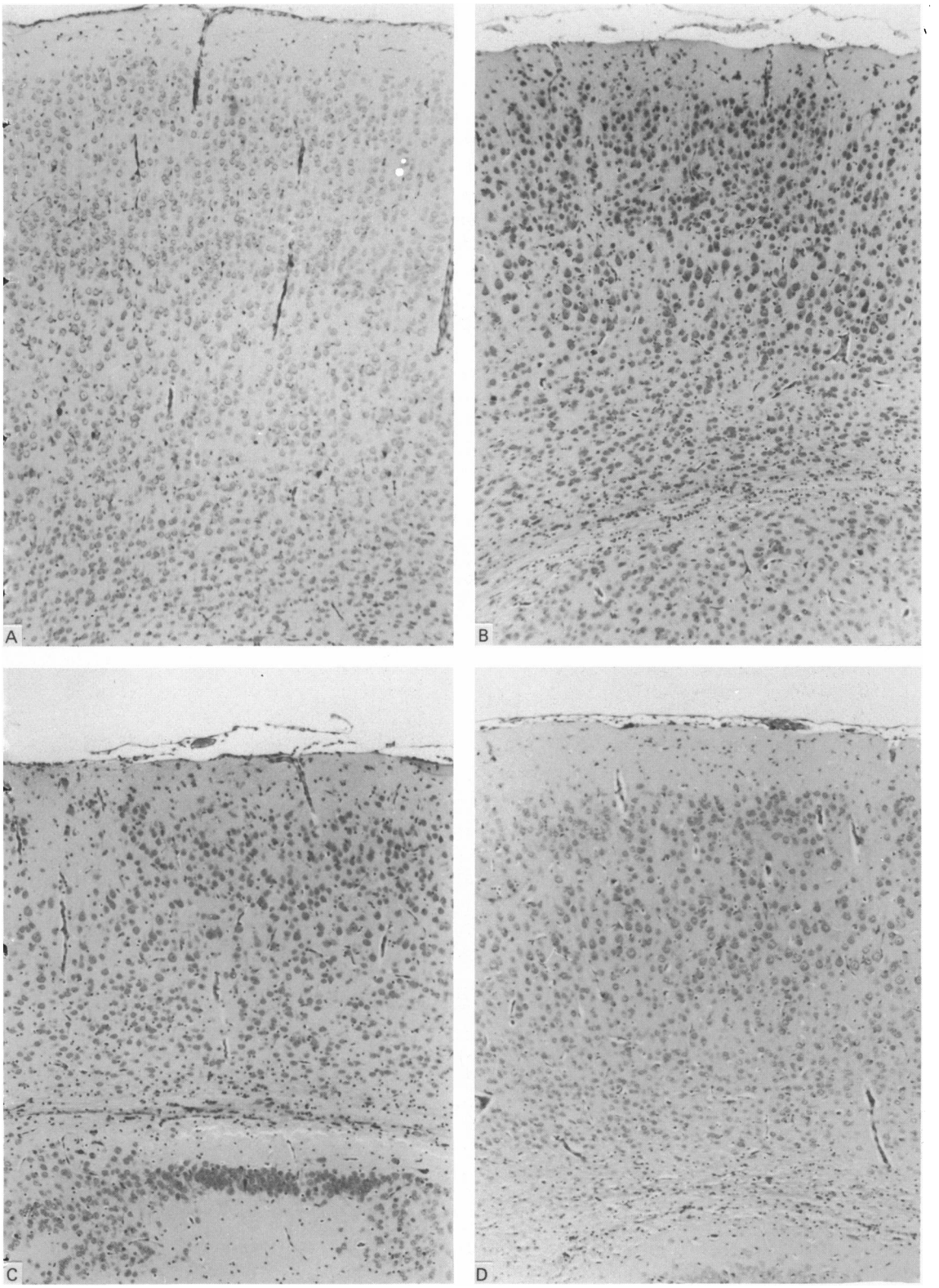
Rats irradiated on the sixteenth gestational day showed a thinner cerebral cortex with abnormal lamination and neuronal nodules located in the middle and superficial regions of the cortex. Dendritic bundles of the inner apical pyramidal cells were present between the cortical nodules (Fig. 1C).

In rats irradiated on the eighteenth gestational day, layers V and VI were normal, the numbers of neurons in layer IV had decreased and the superficial layers were poorly defined with abnormal cell grouping and mal-rotation of neurons (Fig. 1D).

Ectopic germinal rosettes were observed in the telencephalic mantle in embryos at 16 days after conception, following X-irradiation on the fourteenth gestational day (Fig. 2A).

Large subcortical masses were present in rats irradiated on the fourteenth gestational day (Fig. 2B). Ectopic masses attained a larger size at the level of the nucleus accumbens, being smaller in the anterior hippocampal region, and were formed by several nodules separated by ill-defined tracts. Neuronal ectopic nodules were mainly observed in the pyramidal layer of the hippocampus in rats irradiated on the

Fig. 1 (A–D). Representative fields of the cerebral cortex of 30 days old rats. (A) Normal cortex. (B) Rat irradiated on the 14th gestational day; the true cortex and a large subcortical ectopic mass can be observed. (C) Rat irradiated on the 16th gestational day, in which a bizarre cortex and ectopic pyramids are found in the hippocampus. (D) Rat irradiated on the 18th gestational day showing abnormal structure of the superficial region of the cortex. Haematoxylin and eosin. ×63.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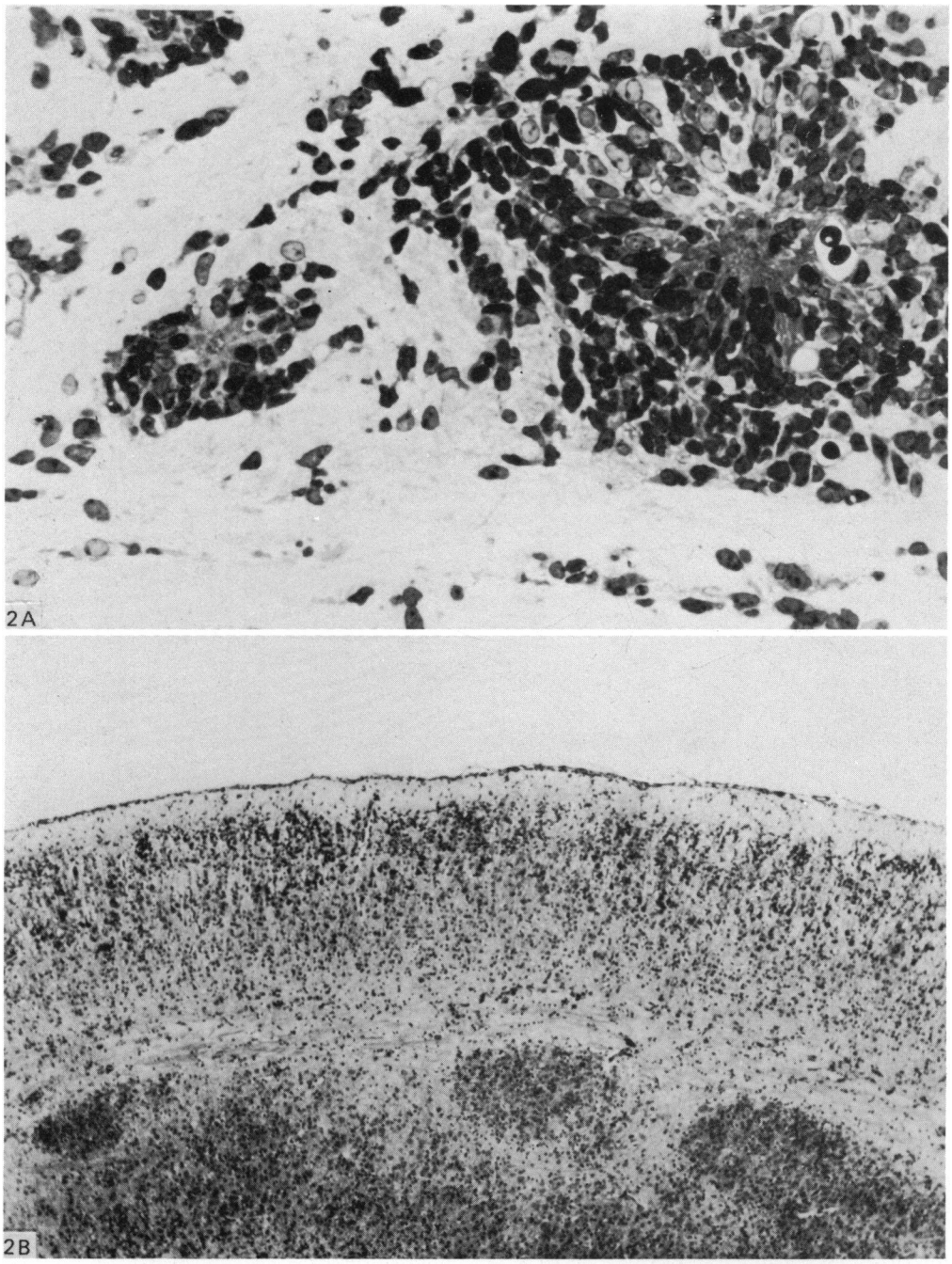

Fig. 2(A–B). (A) Periventricular rosettes in the cortex 18 days after conception in a rat irradiated on the 14th gestational day. Semithin section. Richardson's stain. ×400. (B) Telencephalic mantle of a rat at the 12th postnatal day, irradiated on the 14th gestational day. A large neuronal mass composed of several nodules is observed under the thin cerebral cortex in which a laminated structure may be identified. Cresyl violet. ×40.

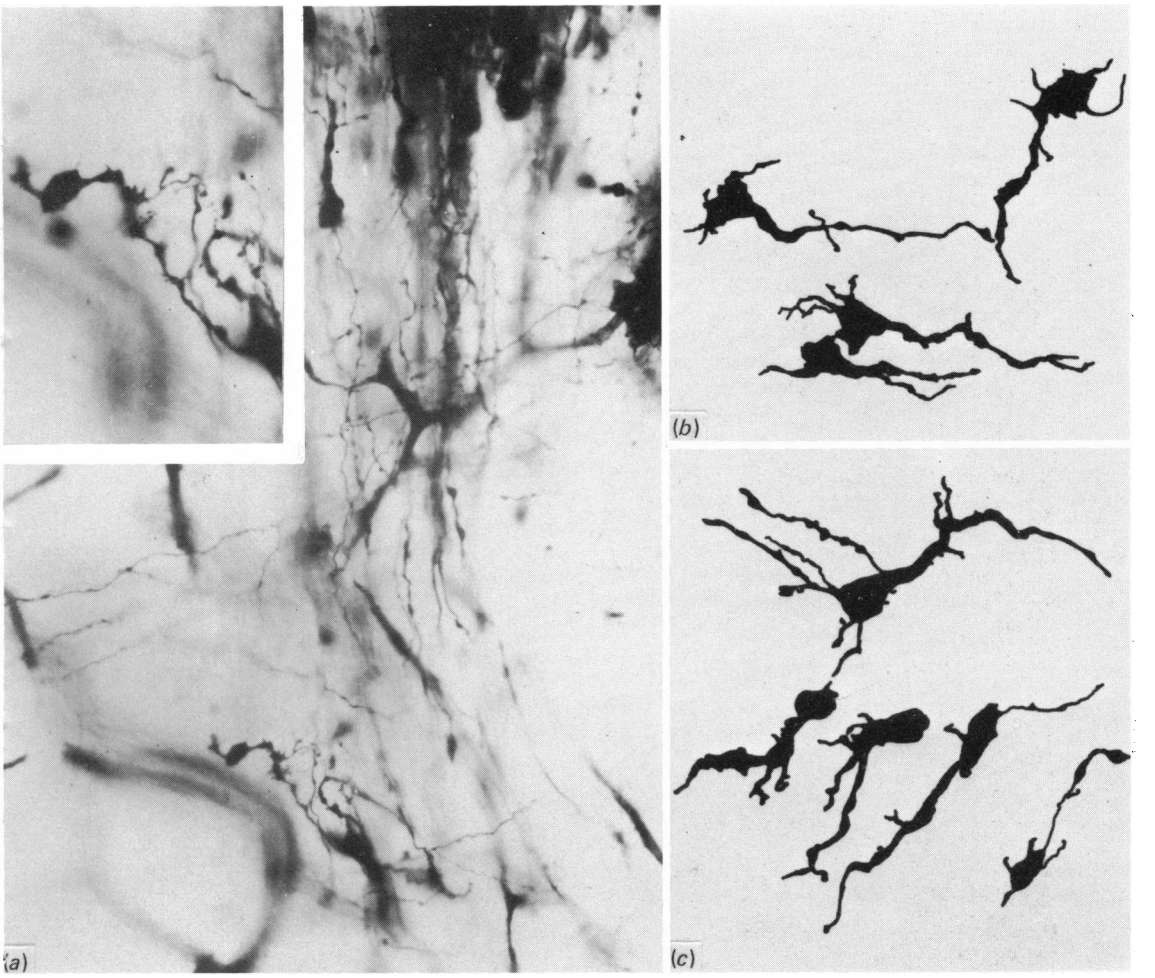

Fig. 3(*a–c*). First stages of neuronal development in ectopic masses. (*a*) A neuron (inset) in the subcortical mass shows dendritic varicosities and growth cones and filopodial processes. Neuroblasts in the cortical mantle are of normal size and configuration in a newborn rat irradiated on the 14th gestational day. × 63; inset × 160. (*b–c*) Camera lucida drawings showing a progressive differentiation of the neurons and cell processes in rats aged 3 and 5 days, respectively. Golgi method. × 160.

sixteenth gestational day. No neuronal ectopic nodules were found in animals irradiated on the eighteenth gestational day (Fig. 1).

Golgi study of the subcortical neuronal masses

Immature features were observed in neurons during the first postnatal days. Most neurons showed monopolar processes with a random orientation; basal dendrites were small and fine. Growth cones and filopodial processes were easily identified (Fig. 3).

Different neuronal types were more easily identified after the first postnatal week. The largest group consisted of pyramidal neurons; small, medium sized and large neurons were intermingled and dendritic bundles could not be identified. Stellate neurons wererandomly distributed as were bipolar and polymorphic neurons (Fig. 4).

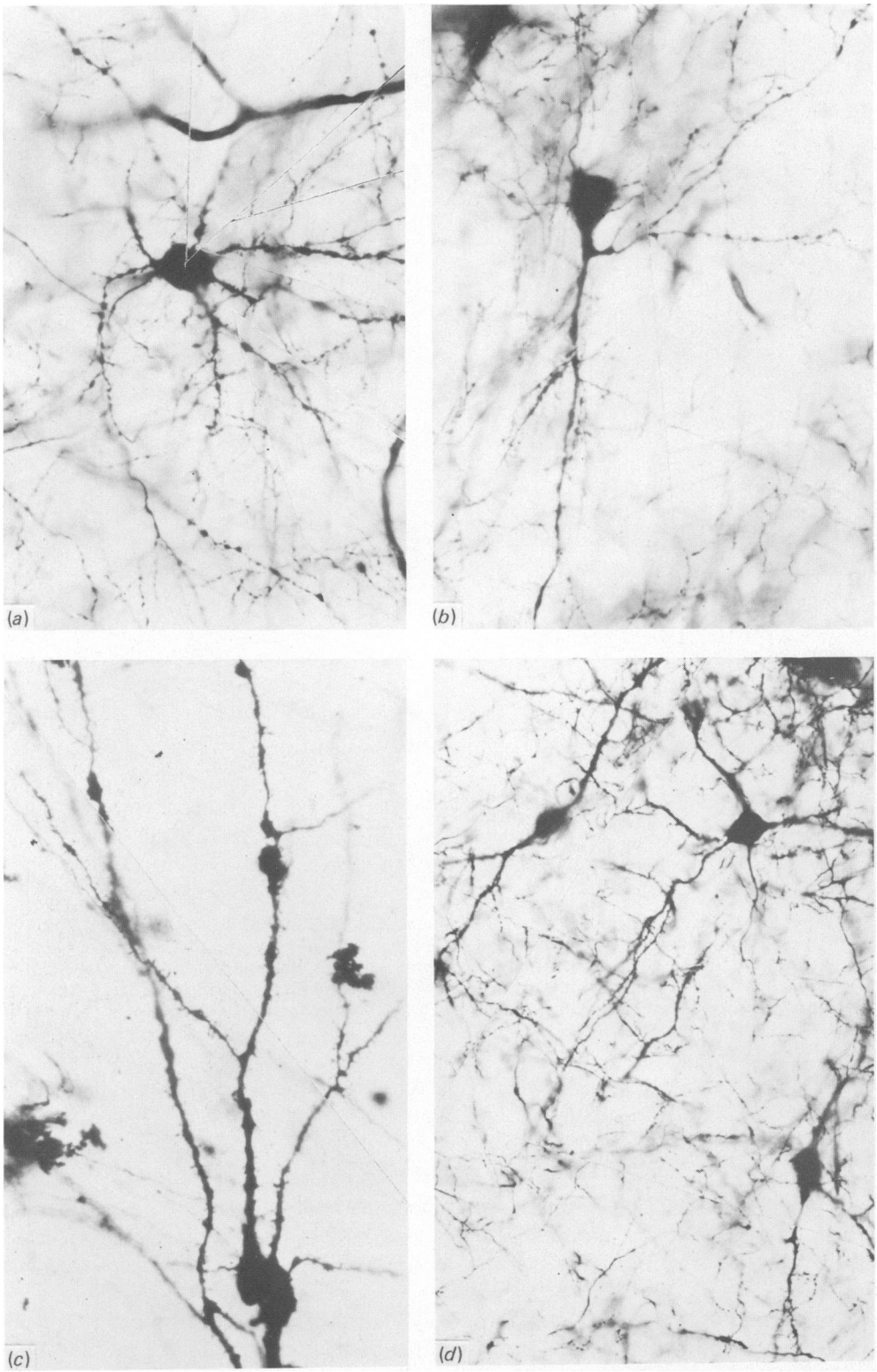

Fig. 4(*a–d*). Different neuronal types in ectopic masses of rats 7 to 10 days old induced by radiation on the 14th gestational day. (*a*) Stellate neuron with dendritic spines. ×320. (*b*) Pyramidal neurons with their apical processes directed inwards. ×320. (*c*) Two pyramidal cells with apposed courses of their apical processes. ×280. (*d*) Bipolar and polymorphic neurons. ×160. Rapid Golgi method.

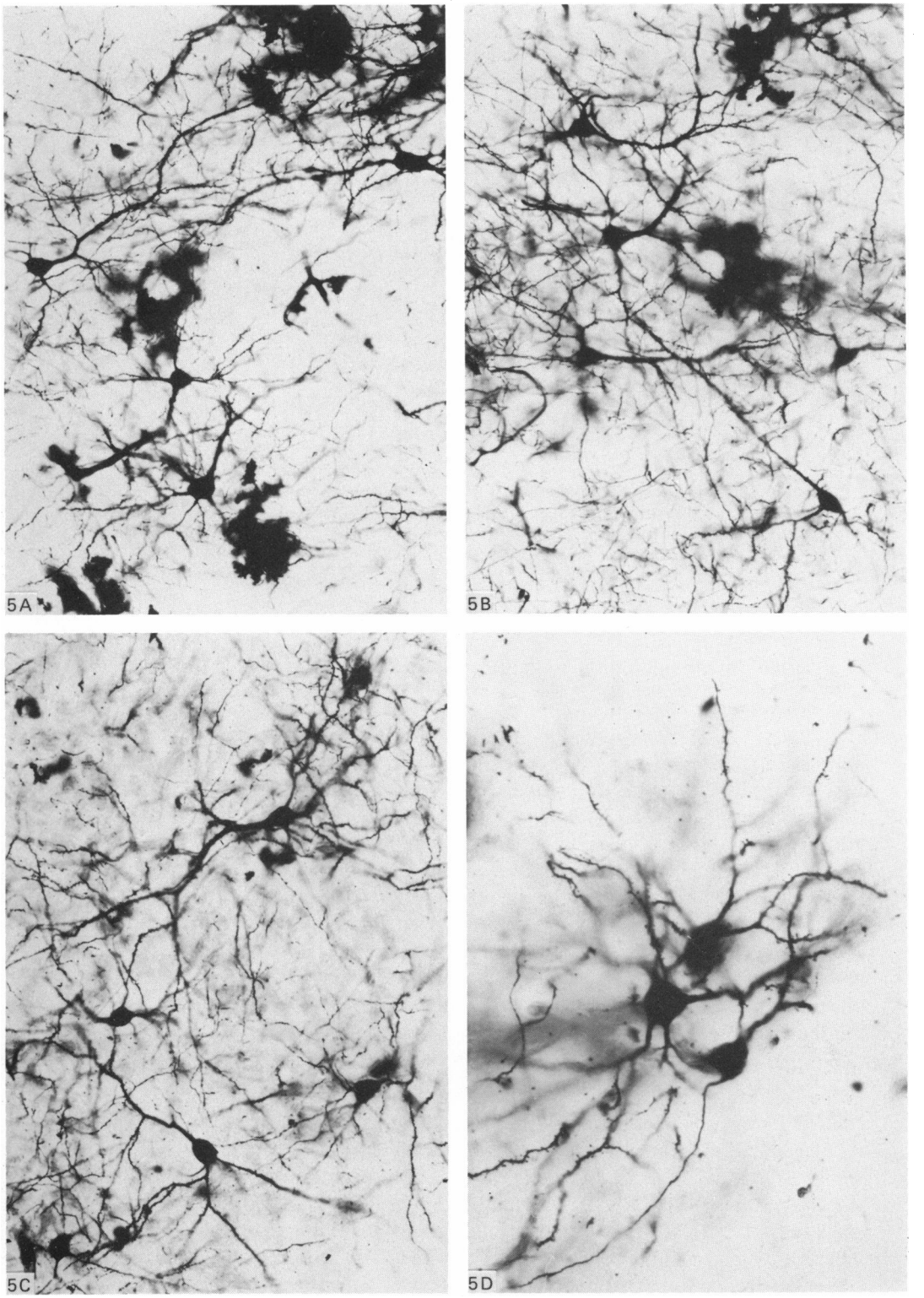

Fig. 5(A–D). Different cell types observed in the subcortical masses of 21–30 days old rats, irradiated on the 14th gestational day. Rapid Golgi method. (A–C) × 160; (D) × 400.

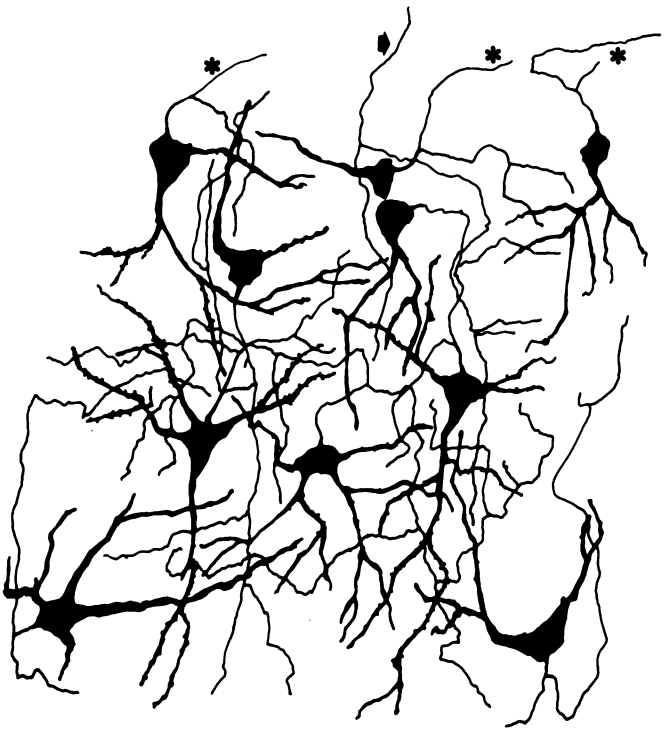

Fig. 6. Camera lucida drawing of a representative field of an ectopic mass, induced by X-irradiation on the 14th gestational day, in which different neuronal types and their axonal branches are represented. Three neurons show efferent axons (asterisk). The arrow indicates an afferent axon. × 220.

Neurons with double dendritic branching were not stained in any section. A disorganised pattern was more evident at low magnification and with camera lucida drawings (Figs. 5, 6).

Intercellular connections in the subcortical masses were difficult to systematise. The axons of stellate neurons branched and extended only within the subcortical masses. The axons of pyramidal neurons coursed and ramified within the malformation but a substantial number reached the subcortical fibres of the white matter. These axons followed two courses, either running in parallel with the fibres of the white matter, where they could be followed for long distances, or penetrating the adjacent cerebral cortex after a short oblique course (Fig. 6).

Golgi studies of the cerebral cortex

The cerebral cortex of rats irradiated on the fourteenth gestational day showed small and medium sized pyramidal neurons in layers II–III, large pyramidal neurons in layer V and polymorphic neurons in layer VI. Horizontal neurons were observed deeply in this layer as in layer VII of normal cerebral cortex (Ferrer & Martinez Matos, 1981). Compared with normal cortex (Fig. 7A), abnormalities were minor and were related to the early distal branching of the apical dendrites of pyramidal neurons, which in the superficial layers showed a stellate-like appearance (Fig. 7B).

The cerebral cortex of rats irradiated on the sixteenth gestational day showed a bizarre organisation; distribution of the neurons was altered, although large pyra-

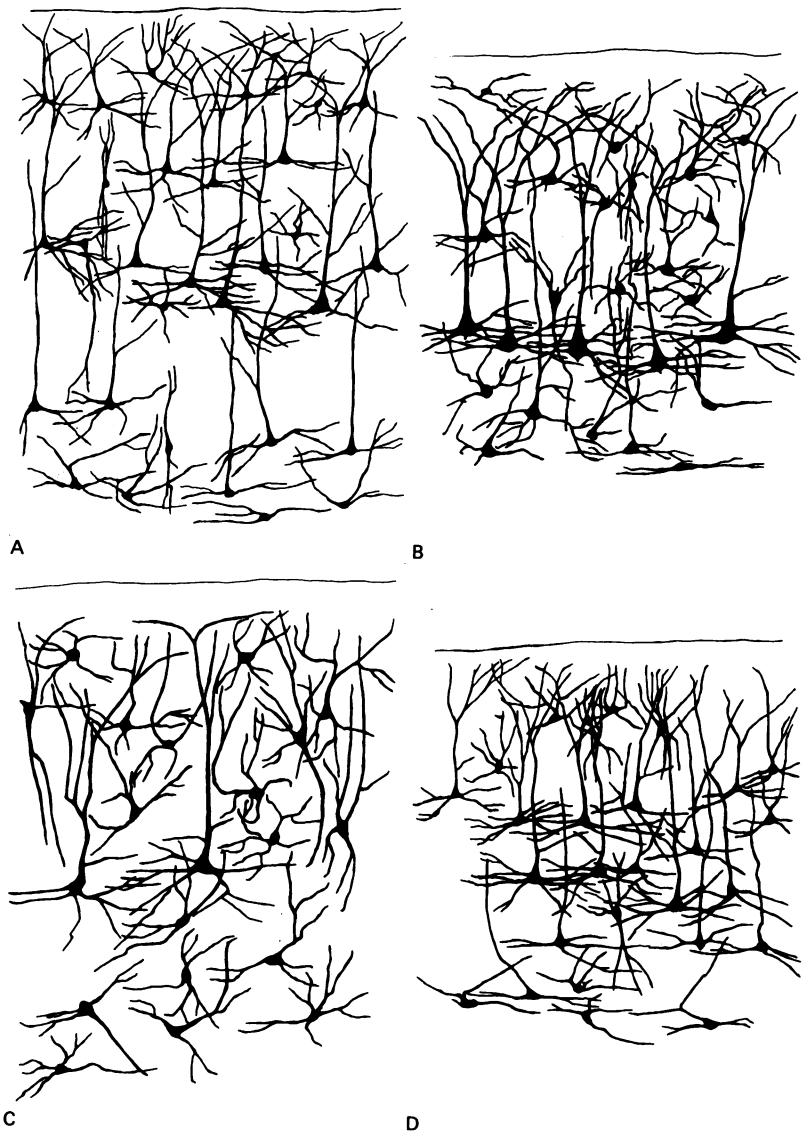

Fig. 7(A–D). Camera lucida drawings based on Golgi sections of the cerebral cortex of normal and irradiated rats. × 80. (A) Normal rat. (B) Cerebral cortex of a rat irradiated on the 14th gestational day. The architectural pattern is essentially normal. (C) Cerebral cortex of a rat irradiated on the 16th gestational day, in which abnormal distribution and bizarre orientation of the neurons may be observed. (D) Cerebral cortex of a rat irradiated on the 18th gestational day, showing abnormal neuronal organisation in the superficial region of the cortex.

midal cells predominated in the medial regions of the cortex. In addition, orientation of the different neuronal types was largely capricious (Fig. 7C).

In the cerebral cortex of rats irradiated on the eighteenth gestational day, abnormal neurons were restricted to the superficial regions, mainly consisting of neurons with an oblique orientation of their apical dendrites, and showing early branching of small and medium sized pyramidal cells (Fig. 7D).

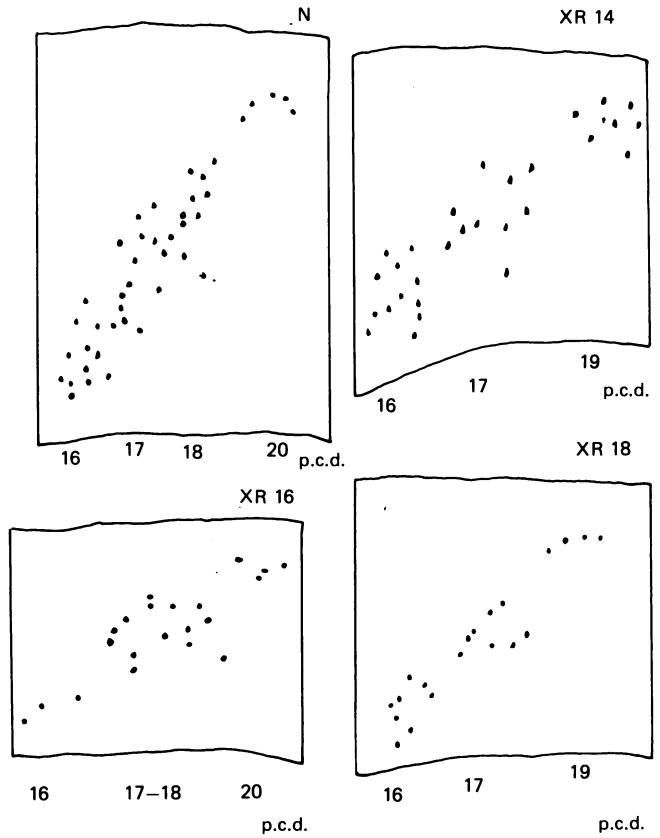

Fig. 8. Autoradiographic studies of sequential neuronal migration to the cerebral cortex after tritiated thymidine injection at various days after conception (p.c.d.). ×40. Note the similar inward–outward gradient in normal rats (N) and in rats irradiated on the 14th (XR14), 16th (XR16) and 18th (XR18) gestational days.

Autoradiographic studies

Neurons labelled with tritiated thymidine on the sixteenth gestational day occupied the deep regions of the cerebral cortex in rats irradiated on the fourteenth gestational day and killed at different postnatal ages. Neurons labelled on the seventeenth gestational day occupied the middle regions of the cortex, while cells labelled on the nineteenth gestational day were those forming the superficial layers of the cortex (Fig. 8).

A similar migratory gradient was observed in rats irradiated on the sixteenth gestational day. Few labelled cells were observed in the deep regions of the cortex after injection of thymidine on the sixteenth gestational day. Neurons were labelled in the medial regions of the cortex after thymidine injection on the seventeenth to eighteenth gestational days. Few neurons restricted to the superficial regions (excluding the molecular layer) of the cortex had incorporated the isotope although widely distributed glial cells were labelled on the twentieth gestational day.

Neurons labelled on the nineteenth gestational day were observed in the superficial layers of the cerebral cortex of rats irradiated on the eighteenth gestational day. Deep and middle regions of the cortex were labelled sequentially on the sixteenth and seventeenth gestational days, respectively (Fig. 8).

No definite conclusions could be drawn about cellular migration in subcortical masses. However, some neurons located peripherally in the nodules of the subcortical masses were labelled at the later gestational ages.

DISCUSSION

Cellular necrosis of germinal cells occurs a few hours after X-irradiation (Hicks *et al.* 1961; D'Agostino & Brizee, 1966; Takeuchi *et al.* 1976; Altman, Anderson & Wright, 1967, 1968), cells in prophase and post-mitotic primitive cells being the most sensitive after 200 rad (Hicks *et al.* 1961). Proliferation of neurons to the cerebral cortex occurs from the fourteenth to the twentieth gestational day in the rat (Berry, Rogers & Eayrs, 1964; Hicks & D'Amato, 1968), and therefore cortical neuronal loss follows X-irradiation during this critical period. In addition, characteristic abnormal patterns of cellular migration and of neuronal organisation are produced by cellular remodelling and impaired cellular recognition (Hicks *et al.* 1959; Hicks & D'Amato, 1966; Dekaban, 1969; Schmahl *et al.* 1979). Large subcortical masses develop from the ectopic germinal rosettes which are formed following X-irradiation on the fourteenth day of gestation, as revealed by current histological and Golgi methods. In such instances, abnormal migration is restricted to the subcortical masses, while migration to the true cerebral cortex, as revealed by sequential labelling with tritiated thymidine, remains unaffected. Cellular structure of the true cerebral cortex shows minor changes and lamination is essentially normal, although the upper layers are ill-defined.

A bizarre cerebral cortex is found following X-irradiation on the sixteenth day of gestation. Cortical lamination is absent and neuronal nodules predominate in the superficial regions of the cortex. Striking abnormalities of cellular distribution and orientation are further demonstrated in Golgi sections (Donoso & Norton, 1982). However, the sequence of cellular migration is surprisingly well preserved, following the normal radial gradient outwards (Angevine & Sidman, 1961; Berry *et al.* 1964; Hicks & D'Amato, 1968; Rakic, 1974). In this case, abnormal neuronal migration is restricted to the small subcortical masses and to the ectopic pyramidal nodules in the hippocampus, while abnormal neuronal organisation dominates the structure of the cerebral cortex.

Similar findings are observed after X-irradiation on the eighteenth day of gestation. Cellular migration to the cerebral cortex is normal, but neuronal organisation is altered in the superficial regions of the cortex.

The present findings indicate that cellular migration and cellular organisation are genetically differentiated mechanisms, although both may be affected separately by X-irradiation.

Complementary data can be obtained from the study of brain malformations induced experimentally by physical (Dvořák, Feit & Juráuková, 1973) or chemical agents (Ferrer, Fabregues & Palacios, 1982).

The ectopic subcortical masses are composed of several types of cortical neurons, including pyramidal, stellate, polymorphic and bipolar cells. However, an organised laminated or columnar structure, integrated by dendritic bundles, is not observed; furthermore, neurons are randomly distributed and capriciously oriented. Autoradiographic studies fail to demonstrate an organised pattern of migration. These results are in contrast with the well documented organised pattern of neuronal migration in the diencephalic region of the rat (McAllister & Das, 1977; Altman &

Bayer, 1978*a*, *b*, *c*; 1979*a*, *b*, *c*). Subcortical masses are then characterised by an abnormal migratory pattern, abnormal organisation but with neuronal differentiation preserved.

The morphological features of the ectopic masses are similar to those observed in transplants of brain tissue in the brain of the rat (Das, 1975; Das, Hallas & Das, 1980; Hallas, Das & Das, 1980; Jaeger & Lund, 1981). They show variable numbers of neuronal types and a rich network of intrinsic dendritic and axonal branches, thus suggesting a complex system of putative intrinsic connections, also observed in certain human gangliocytomas (Ferrer, Isamat & Acebes, 1979; Ferrer, Ribalta, Digon & Acebes, 1983). Additionally, both transplants and ectopic masses show input and output axonal connections, thus indicating integrative functions with other cerebral centres, mainly with the adjacent cerebral cortex.

SUMMARY

Brain malformations are produced after X-irradiation at different post-conceptional ages in the rat. Malformed cortical patterns result from abnormal organisation and capricious orientation of the neurons, while a radical migratory pattern of neuroblasts outwards to the cerebral cortex is preserved in animals irradiated on the fourteenth, sixteenth or eighteenth days of gestation.

Migratory disturbances are restricted to the large subcortical ectopic masses found in rats irradiated on the fourteenth gestational day and to pyramidal ectopic nodules in the hippocampus in rats irradiated on the sixteenth gestational day.

Subcortical ectopic masses develop from ectopic germinal rosettes and are formed by several types of cortical neuron distributed in a stereotyped pattern. The presence of large numbers of intrinsic, afferent and efferent connections are indicative of integrative functions of the subcortical masses.

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