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POST-NATAL ORIGIN OF MICRONEURONES IN THE RAT BRAIN

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MITOTIC neurones are seldom, if ever, seen after birth in the brains of mammals, from which it is commonly concluded that neurogenesis is a pre-natal phenomenon. This conclusion is not often questioned, even though post-natal neurogenesis in some brain structures has been well established for some time. Thus it was recognized in the nineteenth century[1–3] that a class of small nerve cells, the granule cells, shows a high rate of proliferative activity in the cerebellar cortex in young animals. Likewise, good quantitative evidence was obtained some time ago[4] of an increase in the total number of nerve cells in the cerebral cortex of rats up to the 20th day after birth. In this article we shall describe recent autoradiographic evidence from our laboratory which shows clearly that a large proportion of the short-axoned neurones present in various brain structures are formed after birth in the rat.

In these investigations we injected rats systemically with tritiated thymidine. Thymidine is a specific precursor of chromosomal DNA and it is incorporated into cell nuclei only when new DNA is formed by cells preparing for multiplication[5]. When animals are injected with tritiated thymidine, the cells proliferating at the time of injection tend to incorporate the administered radiochemical and they thus become 'tagged'. The radioactively labelled cells are then easily identified with fine-resolution autoradiography. Moreover, by killing animals after varying periods of survival following the injection, a time-lapse record may be obtained of the fate of the originally labelled cells, such as the kinetics of their re-multiplication, and their migration and transformation.

In a pilot study[6] we made stereotaxic lesions in the lateral geniculate body in rats and then injected a small dose of tritiated thymidine into the lesion area. The

Fig. 1. Low-power photomicrograph of a gallocyanin-chromalum-stained autoradiogram of a brain section from a rat injected with tritiated thymidine at the age of 6 h and killed 24 h later. Black dots are the labelled cells. *A*, Ammon's horn; *C*, cortex; *D*, dentate gyrus; *F*, fimbria hippocampi; *P*, choroid plexus; *T*, thalamus; *V*, lateral ventricle. (×68)

animals were allowed to survive for different periods after the injection and the utilization of the injected tritiated thymidine was examined by autoradiography. We found that numerous glia cells were labelled in brain regions structurally or functionally associated with the lesion site. This indicated induced proliferation of glia cells by the brain lesion. However, we found labelled glia nuclei in smaller numbers in areas which could not be related to the traumatized brain area, suggesting the possibility of glial multiplication as a normal phenomenon. In a subsequent study[7] we injected tritiated thymidine systemically into normal adult rats and intraventricularly into adult cats. The examination of this material showed that glia cells proliferate at a low but significant rate in virtually all parts of the normal, adult mammalian brain.

A more surprising finding was that not only the nuclei of glia cells but also the nuclei of occasional neurones were labelled in the brains of adult rats and cats[8]. We were particularly impressed by the labelling of granular nerve cells in the dentate gyrus of the hippocampus[9]. In all the animals (mature adults included) and all the sections examined a few labelled granule cells were always encountered. Since the granular layer of the dentate gyrus is essentially devoid of the nuclei of glia cells, the labelling in this region could be attributed only to uptake of thymidine by the nuclei of the granule cells.

Accordingly, in our next investigation[10] we undertook to investigate in detail the nature and significance of DNA metabolism in the dentate gyrus of the hippocampus. Rats of varying ages (from neonates to adults) were injected systemically with tritiated thymidine and the animals were permitted to survive for selected periods after the injection. In addition, to obtain collateral material for histological evaluation the brains of a large series of non-injected rats of different ages were prepared for microscopy.

This study showed that the number of labelled cells in the dentate gyrus is very high in neonates and infants, and declines to a low but appreciable level in adults. Cell counting in collateral material showed that the number of differentiated granule cells in homologous sections increases six-fold from birth to 3 months of age, suggesting that the autoradiographic evidence may have reflected hippocampal neurogenesis. Counter-indicating this conclusion was the fact that mitotic cells were seldom encountered in the dentate gyrus. In further examination

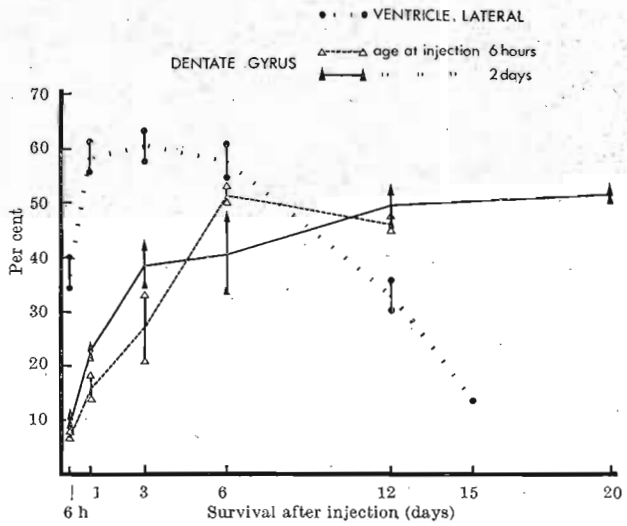

Fig. 2. Percentage of labelled cells in the germinal lateral ventricle and in the dentate gyrus as a function of survival time after injection

of this material we were impressed by the fact that in the brains of neonate and infant rats there is an extensive ependymal and sub-ependymal germinal matrix around the lateral ventricle, composed of cells with darkly staining nuclei and occasional mitotic ones. Such darkly staining cells were also seen in the fimbria, and they were particularly numerous in the dentate gyrus in the brains of the younger animals. Quantitative work showed that the number of cells and the areal extent of the lateral ventricle declined rapidly after birth, with a transient rise at about 15 days. A delayed, rapid rise could also be seen in the number of small cells in the dentate gyrus, showing a decline also at 15 days of age. The decline in the latter was accompanied by a corresponding increase in the number of differentiated granule cells with a peak at about 3 months of age. On the basis of these observations we postulated that undifferentiated cells or neuroblasts multiply at the lateral ventricle, migrate from there by way of the fibrous tracts of the hippocampus to the dentate gyrus and become differentiated there in the course of development into granule cells.

The hypothesis of the previous investigation was confirmed in an extensive subsequent series of experiments in which groups of neonate, infant, adolescent and adult rats were injected systemically with tritiated thymidine (10 μc./g body-wt.; specific activity 6·7 c./mmole; radiochemical dissolved 1 mc./ml. in isotonic saline). The animals were allowed to survive for 6 h, 1, 3, 6, 12, (15), 20, 30, 60, 120 and 180 days after the injection. In this article we shall deal merely with some of the results obtained in the rats injected as neonates, particularly at 2 days of age. (A detailed evaluation of the entire experimental material is in preparation.)

In neonates injected with tritiated thymidine (Fig. 2) 35–40 per cent of the cells in the ependymal and subependymal layers of the lateral ventricle were labelled in the animals killed 6 h after the injection. The rate of cell labelling, with concomitant dilution of labelling within cells, rose to about 60 per cent 24 h after the injection. With prolonged survival after injection there was a considerable dilution of the label within the cell nuclei and, after 6 days of survival, a reduction in the total number of labelled cells. This suggested a continued

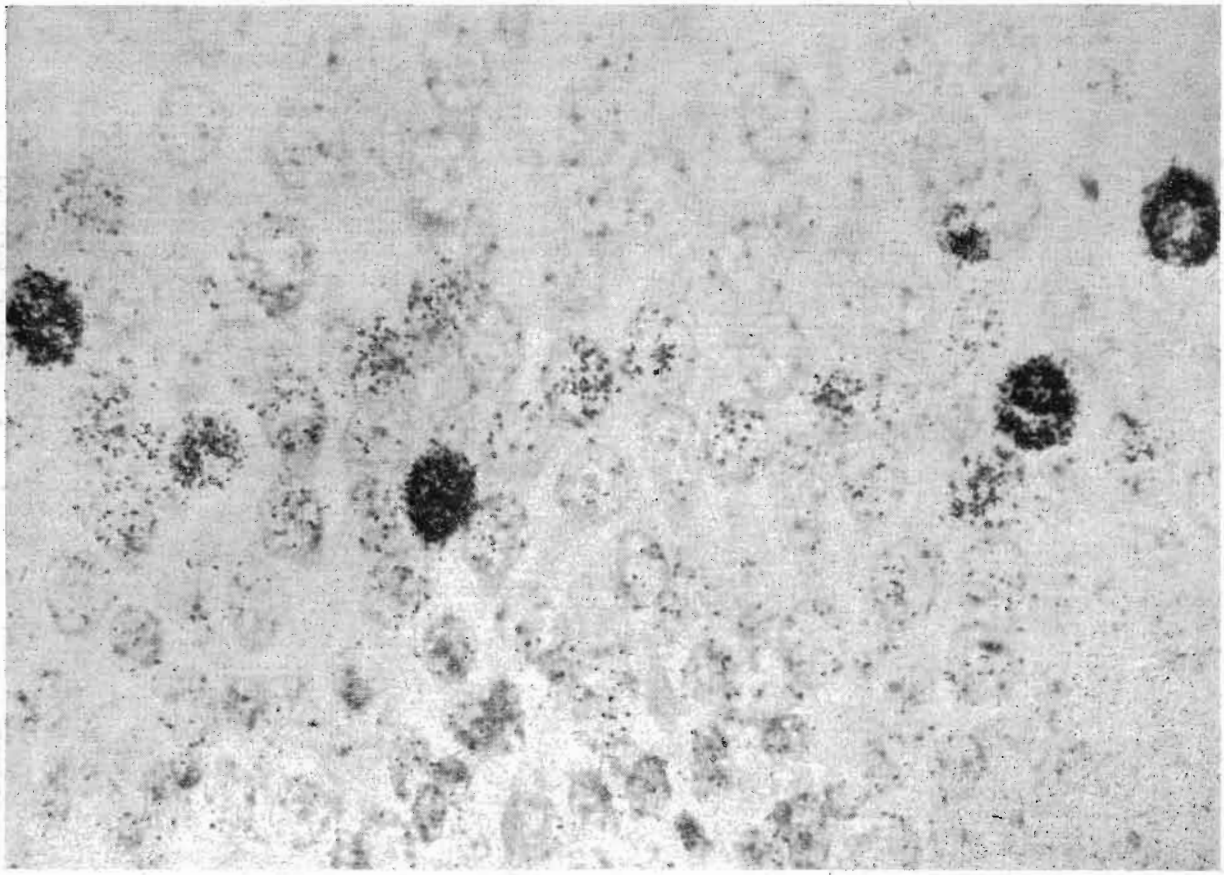

Fig. 3. High-power photomicrograph of a gallocyanin-chromalum-stained autoradiogram of granule nerve cells in the granular layer of the dentate gyrus. The animal was injected with tritiated thymidine at 2 days of age and survived for 20 days after the injection. (×880)

multiplication of the initially labelled cells and their migration to other brain regions. Cell labelling was initially low in the dentate gyrus (less than 10 per cent), and in the animals with short survival after injection these were typically small cells with darkly staining nuclei, situated in the plexiform layers surrounding the ill-developed granular layer. The number of labelled cells increased to about 45–50 per cent by the 12th day after injection, by which time the majority of labelled cells have migrated into the thickening and expanding granular layer, and many of the labelled cells had the appearance of differentiated granule cells.

The distribution and differentiation of labelled cells in the granular layer followed a regular pattern. In animals killed 20 days after the injection, the top row of cells in the granular layer (the cells in intimate contact with the superficial plexiform layer) were typically unlabelled. These we consider the 'oldest' or earliest-differentiating cells of the dentate gyrus, derived from cells which multiplied before the administered thymidine became available. Below this row of unlabelled cells were many intensely labelled cells. Absence of appreciable label dilution indicated that these cells ceased to multiply soon after the injection; that is, they represent the 'oldest' of the post-natally formed cells. Moving from the superficial to the basal rows of granule cells intensity of cell labelling decreased gradually, with many unlabelled cells to be found in the basal rows of cells; the latter represent the 'youngest' of the granule cells. Cell size and cellular differentiation showed the reverse pattern. The cells in the superficial row tended to differentiate first and were among the largest; the basal cells were smaller and often undifferentiated.

The post-natal genesis of granule cells is not an exclusive characteristic of the dentate gyrus of the hippocampus. Our results show clearly that the bulk of the granule cells in the granular, mitral and glomerular cell layers of the olfactory bulb are formed post-natally. In this region neuroblasts proliferate at a high rate around the wall of the olfactory ventricle (Fig. 4), then move out and invade gradually the layers of the olfactory bulb, where they presumably differentiate. A large proportion of the granule cells of the internal granular layer of the cerebellar cortex (and to a lesser extent the Golgi cells and basket cells) are formed post-natally. This occurs both by local multiplication and through the migration of cells multiplying in the external granular layer (which is a germinal matrix that disappears in the rat at about 20 days of age)

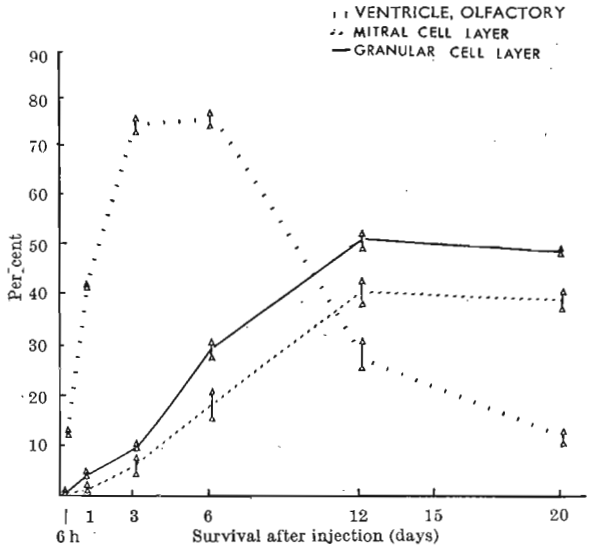

Fig. 4. Percentage of labelled cells in the germinal olfactory ventricle and in the mitral and granular cell layers of the olfactory bulb in rats injected with tritiated thymidine at 2 days of age

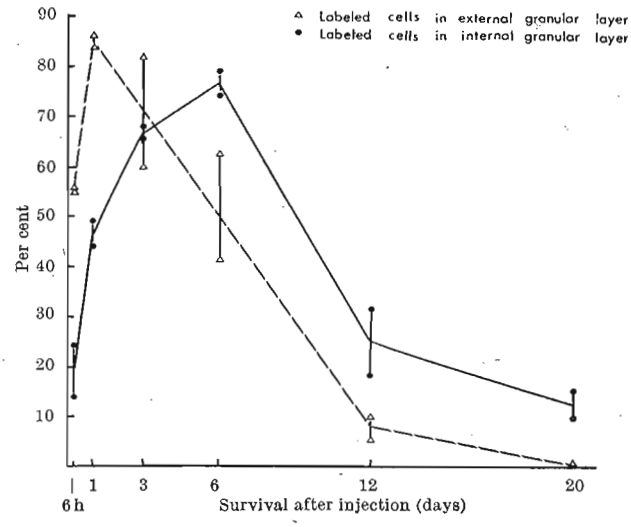

Fig. 5. Percentage of labelled cells in the external and internal granular layers of the cerebellum

into the internal granular layer (Fig. 5). Similarly, the granule cells of the granular layer of the ventral cochlear nucleus are largely formed post-natally through migration of cells multiplying in the lateral recess of the fourth ventricle. It is very likely, though not conclusively proved, that many of the granular or stellate cells of the cerebral cortex also are of post-natal origin.

In the same structures in which a large proportion of the granule cells became labelled with tritiated thymidine, the nuclei of neighbouring large neurones remained typically unlabelled. The large mitral cells in the olfactory bulb were never seen with labelled nuclei, nor were the nuclei of the polymorph cells of the dentate gyrus, of the pyramidal cells of Ammon's horn, or the Purkinje cells of the cerebellum ever labelled. In general, large or long-axoned neurones (occasional ones in the cortex excepted) do not incorporate tritiated thymidine injected into neonates, whereas a considerable number of neuroblasts which afterwards differentiate into small, short-axoned neurones predictably utilize tritiated thymidine. From these findings we may conclude that there is a radical difference, from the developmental point of view, between the short-axoned neurones or microneurones[11] of the central nervous system and the long-axoned neurones or macroneurones. We shall not speculate here about the possible functional significance of the post-natal origin of the majority of microneurones. Elsewhere[12] we have made the suggestion that the pre-natally formed afferent, relay and efferent macroneurones represent the invariant or fixed components of the central nervous system, whereas the interposed microneurones, which develop post-natally as the animal comes to face and respond to its varied external environment, could be its modulatory and plastic elements.

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