Postnatal neurogenesis in the caudate nucleus and nucleus accumbens septi in the rat

In recent years various investigators using tritiated thymidine autoradiography have established that in mice and rats, neurogenesis in the cerebellum[3-5,10,11,17], hippocampus[3-6] and olfactory bulb[3,4] continues for a considerable period during postnatal development. These studies were concerned with neurogenesis of small neurons, primarily granule cells, in these three neural structures. In our past work[1], attention was drawn to the presence of some large labeled neurons in the cortex of rats and cats which were injected with [^{3}H]thymidine, either soon after birth or in early adulthood. The distribution of these large neurons did not reveal any discernible pattern and they were described as randomly distributed. However, evaluation of other regions of the rat brain revealed that a considerable number of large neurons in the caudate nucleus and the nucleus accumbens septi are formed after birth, and that these postnatally-forming neurons present a definite pattern of distribution. In this report some details are provided regarding the genesis and distribution of these large neurons.

Laboratory-bred Long–Evans hooded rats were used for this study. Only those animals were used in this study which had a gestation period of 22 days. At each stage of postnatal development at least two pups from different litters were administered [^{3}H]thymidine, 10 μCi/g body weight, intraperitoneally (specific activity: 6.7 Ci/mM, 1 mCi of radiochemical dissolved in 1 ml of isotonic saline). The 0-day-old animals received a total of 60 μCi [^{3}H]thymidine each within 6 h after their birth; 2-day-old animals 70 μCi each and 6-day-old animals 120 μCi each. The radiochemical was given in one injection to every animal. Two additional animals were used for cumulative injection of [^{3}H]thymidine. They received the radiochemical (10 μCi/g body weight) on the 1st, 3rd, 5th, and 7th days after birth. These two animals received a total of 360 μCi of the radiochemical. All these animals were kept for 120 days, they were perfused with 10% neutral formalin and their brains were removed. After further fixation for 2–3 weeks, the brains were processed for histology and cut serially at 7 μm thickness. The sections were prestained with gallocyanin–chromalum and treated for autoradiography using the dipping technique. The slides were kept in light-proof boxes at 5°C for 13 weeks. At the end of this exposure period the autoradiograms were developed and processed in the routine manner[2].

For microscopic evaluation, several sections were scanned bilaterally, and the labeled neurons were counted at each level of the caudate nucleus and the nucleus accumbens septi using De Groot coordinates[9]. The total number of labeled neurons thus established was reduced to an average number of labeled neurons per section.

Fig. 1 shows the distribution of labeled neurons at different levels of the caudate nucleus along the antero-posterior axis in animals injected on different days after birth. The maximum number of labeled neurons is found in the animals that were injected cumulatively on 4 days, that is on the 1st, 3rd, 5th, and 7th days. This cumulative labeling on 4 days, each separated by an interval of one day, provides a conservative estimate of the number of postnatally-formed neurons during this period

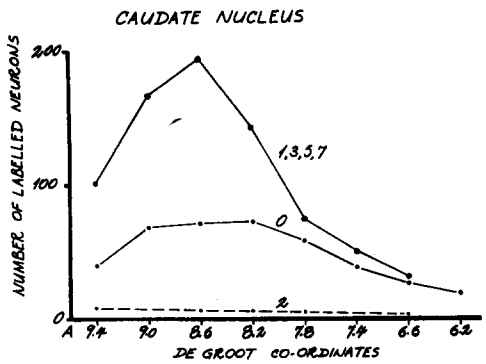

Fig. 1. Average number of labeled neurons in the caudate nucleus at various levels along the antero-posterior axis. Each point represents an average number of labeled neurons present bilaterally in one section. Different levels are indicated in terms of stereotaxic coordinates established by De Groot. 1,3,5,7, Animals injected on the 1st, 3rd, 5th and 7th days cumulatively; 0, animals injected within 6 h after birth; 2, animals injected on the 2nd day postnatally.

because [^{3}H]thymidine is available for a short period only after each injection. Animals that were injected with a single dose on day 0 (within 6 h after birth) show the next highest number of labeled neurons. The animals injected on the 2nd day after birth show a very small number of labeled neurons and those injected on the 6th day show none at all. This suggests that the majority of the labeled neurons seen after cumulative labeling are the result of utilization of [^{3}H]thymidine by the precursors of large neurons that were preparing to divide on the day and the day after birth. More specifically, neurogenesis of the neurons seems to be relatively moderate on the day of birth, while on the following day (day 1), neurogenesis increases to a high magnitude. Following this upsurge, the division of the precursors of large caudate neurons declines very fast on the 2nd day and subsequently it ceases altogether.

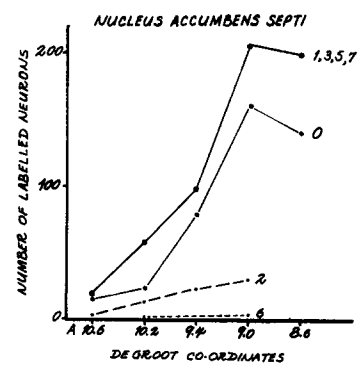

Fig. 2. Average number of labeled neurons in the nucleus accumbens septi at various levels along the antero-posterior axis. Each point represents an average number of labeled neurons present bilaterally in one section. Different levels are indicated according to the stereotaxic coordinates determined by De Groot. 1, 3, 5, 7, Animals injected on the 1st, 3rd, 5th and 7th days cumulatively; 0, animals injected within 6 h after birth; 2, animals injected on the 2nd day; 6, animals injected on the 6th day postnatally.

An analysis of the distribution of labeled neurons shows that they are maximally concentrated in the anterior portion of the head of the caudate nucleus, which is bounded within De Groot coordinates A9.4 and A7.8 (Fig. 1). As we proceed caudally the number of labeled neurons declines rapidly. This gradient is indicative of the direction of growth of the caudate nucleus in relation to the lateral wall of the lateral ventricles.

In the case of the nucleus accumbens septi, also, the largest number of labeled large neurons is seen following cumulative injections of [^{3}H]thymidine on the 1st, 3rd, 5th, and 7th day postnatally (Fig. 2). But unlike the caudate nucleus, the nucleus accumbens septi appears to receive its large bulk of postnatally-forming neurons on the day of birth (0 day). Many labeled neurons are obtained by injecting the animals on the 2nd day, and a small number by injecting on the 6th day. These findings indicate that in the nucleus accumbens septi, unlike the caudate nucleus, large neurons are formed over a protracted period, up to the 6th day after birth. Furthermore, these data suggest that in the case of the nucleus accumbens septi there is no sudden burst of neurogenesis on the 1st day, instead neurogenesis takes place at a steady high rate on the day of birth and on the 1st day and subsequently declining to a slower rate.

The highest concentration of labeled neurons in the nucleus accumbens septi is seen in the caudal region, which lies between De Groot coordinates A9.4 and A8.6. It is between these two levels that the caudate nucleus also has its highest concentration of labeled neurons.

As shown in Fig. 3, most of the labeled neurons are found in those regions of the two nuclei which are close to the lateral and ventral aspects of the lateral ventricles. Occasionally in the medial and lateral regions of the caudate nucleus, a few scattered labeled neurons are seen. This topographic pattern of distribution of the labeled neurons suggests that the postnatally-forming neurons in the caudate nucleus arise from the neuroepithelium along the lateral wall of the lateral ventricles and those in the nucleus accumbens septi arise from the neuroepithelium at the ventral aspects of the lateral ventricles.

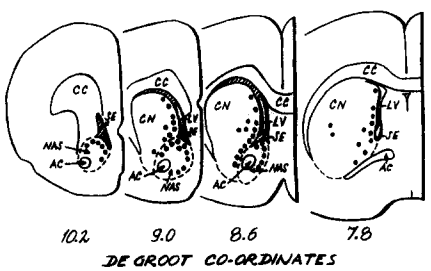

Fig. 3. Schematic presentation of the distribution of labeled neurons in the caudate nucleus and the nucleus accumbens septi at various levels antero-posteriorly. This figure shows differences in density of distribution of the labeled neurons at various levels, relative proximity of the labeled neurons to the subependymal layer of the lateral ventricles and presence of the subependymal layer in the adult rat brain. AC, anterior commissure; CC, corpus callosum; CN, caudate nucleus; LV, lateral ventricle; NAS, nucleus accumbens septi; SE, subependymal layer.

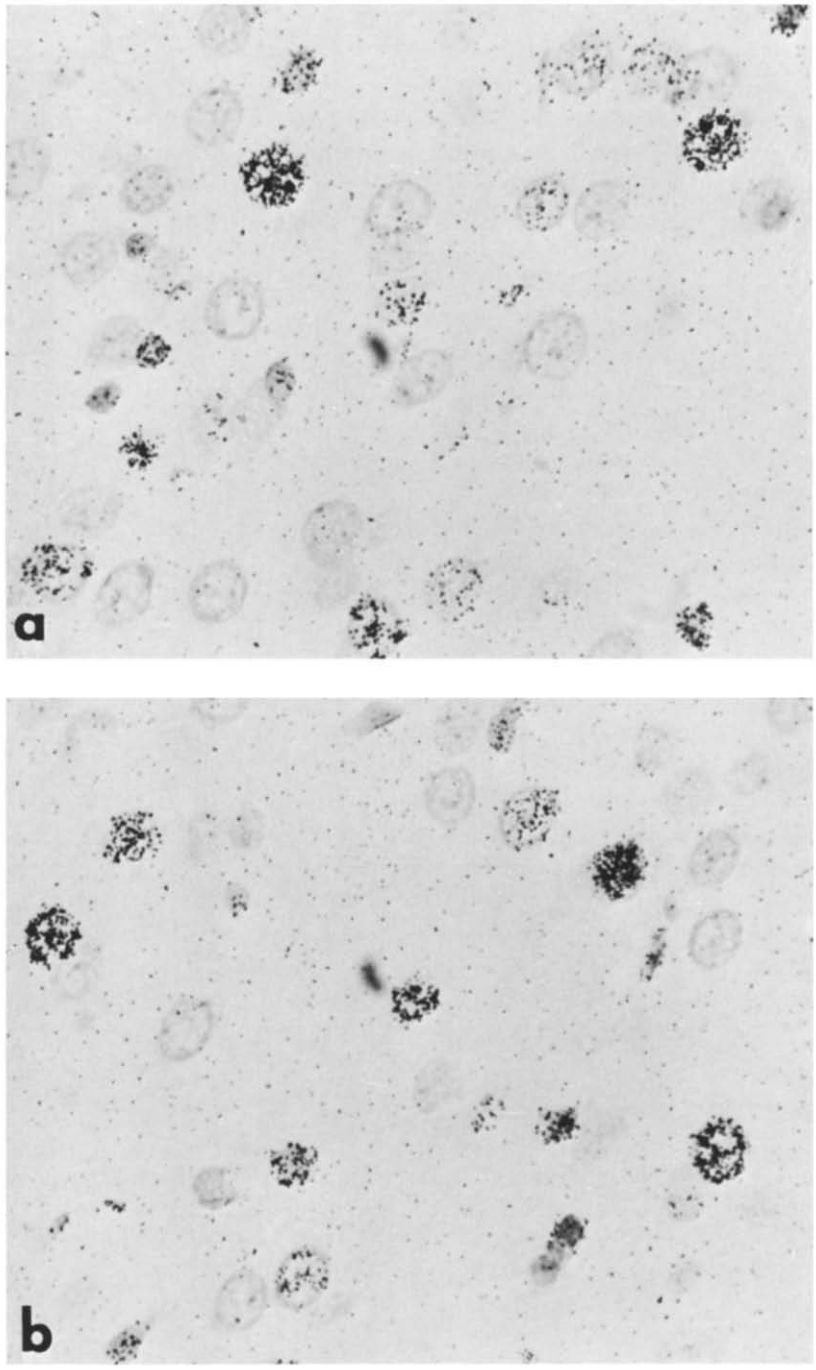

Fig. 4. Labeled neurons in the caudate nucleus (a), and in the nucleus accumbens septi (b), in the animals which were injected with [^{3}H]thymidine cumulatively on the 1st, 3rd, 5th and 7th days postnatally. 1,650 ×.

The labeled neurons in the caudate nucleus and the nucleus accumbens septi show variable intensity of labeling (Fig. 4). It may be due either to unequal availability of [^{3}H]thymidine to the precursors of neurons at the time of its utilization, or differences in the number of mitoses that the precursors of different neurons had to undergo before giving rise to the final neuroblasts. Our previous studies on postnatal neurogenesis in rats show that a dose of 10 μCi/g body weight administered intraperitoneally is more than adequate to overcome regional differences in the availability of the radiochemical in the brain. In the present investigation the latter possibility of differences in the number of mitoses of the precursors of neurons seems to be the case. It means that those precursors of neurons which had only one or at best two mitoses to undergo gave rise to those neuroblasts which differentiated into the intensely labeled neurons. Whereas, those precursors of the neurons which had to undergo a higher number of mitoses before giving rise to the final neuroblasts yielded lightly labeled neurons. This suggests that intensely labeled neurons are early formed and lightly labeled neurons are of the later origin. This observation further supports the conclusion that neurogenesis of these large neurons in the two neural structures is protracted over the first few days after birth.

In the telencephalon, the caudate nucleus, putamen and the nucleus accumbens septi are the earliest structures to be formed[7,8,13]. These neural structures show the presence of acetylcholinesterase activity in very early embryonic stages, suggesting their early enzymatic differentiation[12,14]. In the context of these facts the postnatal origin of large neurons in the medial aspects of the caudate nucleus and in the caudal region of the nucleus accumbens septi acquires a special significance. Cytologically, in Nissl as well as Golgi preparations, the postnatally-forming neurons in the two nuclei are indistinguishable from the other neurons of these structures. The perikarya of these neurons are medium-sized and round in shape, the pale nuclei are surrounded by very thin cytoplasm, which does not have distinct Nissl bodies. In Golgi preparations these neurons show a radiating pattern of dendritic arborization and very dense distribution of dendritic spines on dendrites[15,16]. Despite the cytological identity, the postnatally-forming neurons are distinctly different from the rest of the neurons in the two nuclei, at least in their time of origin. Does postnatal neurogenesis in the caudate nucleus and the nucleus accumbens septi represent the tail-end of neuroembryogenesis of these two neural structures? Or do the subregions of these two nuclei, which are composed of postnatally-formed neuronal elements, have some special anatomical and functional significance? Some of our studies in progress will help answer these questions in the future more adequately.

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