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Postnatal development of the somatosensory thalamocortical projection in the rat and rabbit — a combined retrograde transport and stereological comparative study

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A comparative quantitative study of the somatosensory thalamocortical connections in the rat and rabbit, labeled with the fluorescent retrograde tracer Fluoro-Gold (FG), was conducted by means of unbiased stereology. FG was injected into the primary somatosensory cortex of the rat and rabbit in different age groups from P0 to P180 (P-postnatal day). The numerical density of retrogradely labeled the ventroposterolateral (VPL) projection neurons was analyzed. A significant decrease in this parameter was observed during the first two weeks of postnatal life in both studied species. Changes of the neuropil volume and selective elimination of early cortical connections stemming from the VPL may possibly cause this process. A withdrawal of axon collaterals from the expanded cortical sites as well as apoptosis (existing both in the VPL and parietal cortex) contribute to a decrease in the numerical density.

Our observations allow us to conclude that the thalamocortical somatosensory connections established before the birth undergo significant quantitative changes in both studied species during the first two weeks of postnatal life and this period seems to be crucial for maturation of the thalamocortical loop.

key words: thalamocortical connections, retrograde transport, fluorescent tracer, stereology, somatosensory thalamus, rat, rabbit

INTRODUCTION

The function of the central nervous system depends on the establishing of highly specific interconnections between neurons distributed across widely separated brain regions. Generally, ascending somatosensory information in mammals is relayed to the primary somatosensory cortex through the ventral posterior nucleus (VP). The VP consists of two subdivisions: the ventroposterolateral (VPL) and ventroposteromedial (VPM) nuclei. The former receives tactile inputs from the trunk, limbs and the tail via the dorsal column nuclei and the spinal cord and transmits them to the somatosensory cortex.

The input to the somatosensory cortex from specific thalamic nuclei has been long recognized as a potent force for determining developmental events in the cerebral cortex and for shaping responses of cortical neurons in the adult brain [2]. Although prenatal developmental patterns of thalamocortical synaptic connectivity have been described in the litera-

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ture on the subject, the growth of thalamocortical fibres and the process of synaptogenesis continue to call for intense research efforts [6].

Despite a huge amount of qualitative data on the problem above [5], precise quantitative data is short, especially that on the rabbit. The period in which the establishment is seen of functional connections between the end-organs of the thalamus and cortex, is of various length in various species and does depend on the degree of advanced intrinsic organization on the thalamic level. Moreover, in the rat VPL, in contrast to the rabbit, no local circuit neurons exist. Therefore, it seems interesting to compare the course of developmental changes of thalamocortical connections originated from the VPL in the rat and the rabbit.

MATERIAL AND METHODS

The study embraced 30 Wistar rats put into six age groups (P0, P7, P14, P21, P45, P180) and 32 New Zealand rabbits classed into eight age groups (P0, P7, P14, P21, P60, P90, P120, P180). The care and treatment of animals were in accordance with guidelines for laboratory animals established by the National Institute of Health and the local ethics committee. The method of retrograde axonal transport of fluorescent tracer Fluoro-Gold was used for the study. The operation procedure and histological protocol were previously described in detail [3]. The amount of tracer used and that of stereotaxic coordinates were adjusted to the brain size (age of the animal). The diffusion zone surrounding the injection site occupied all the layers of the cortex, but did not enter the white matter. Coronal serial sections were studied by means of a fluorescent microscope BX-51 (Olympus, Japan) equipped with the UV-filter system providing an excitation wavelength of 365 nm.

The numerical density of retrogradely labeled neurons in VPL was estimated by means of the optical dissector method. The C.A.S.T. grid system (Olympus, Japan) utilizing a microscope BX-51 (Olympus, Japan) was applied. The results for all age groups were compared using ANOVA with the posthoc LSD test (the least significant difference test).

RESULTS AND DISCUSSION

The distinct projection zone occupying the ventroposterolateral thalamic nucleus was observed in both studied species (Fig. 1). During the postnatal development, both in the rat and the rabbit, a significant decrease was seen in the numerical density of retrogradely labeled VPL neurons. The decrease in this parameter in the rat was 25% and 34% during the first and second week, respectively. In the rabbit, in comparison with rat, a two-fold decrease in the numerical density was found during the first week, whereas during the second week the decrease amounted to 43%. After this critical two weeks long period, the density did not change significantly (Table 1).

The decrease in the density of projection neurons in the VP may be a result of neuropil volume increase [4, 7, 10]. Consequently, a higher decrease seen in the rabbit vis a vis that in the rat is probably caused by a more dynamic increase in the VPL volume in the former species.

Another reason for the decrease in the density of projection neurons is a naturally occurring cell death in the early postnatal period [9]. Due to the tight reciprocal relationships of the thalamus and the cortex, the role of the latter in this process can be hardly underestimated. VPL neurons are generated already in prenatal life. The transient population of subplate cells, forming a particular framework for both corticofugal and corticopetal fibres, disappears in the process of apoptosis, thus leading to the death of some VPL neurons and the loss of their temporary targets. Consequently, a decrease manifests in the intensity of somatosensory projection. Then, during the first days of postnatal life, the laminar structure of the cortex differentiates. Apoptosis in the cortex (appearing later than in the thalamus [8] and maintained for some time) causes a decrease in the amount of relay VPL cells.

Thalamic axons undoubtedly play the most important role in determining morphological specifi-

Table 1. The numerical density of retrogradely labeled neurons in VPL. Data are given as mean values (+SD)

Age group	Rat [mm^{-3}]	Rabbit [mm^{-3}]
P0	93243 ± 9647	123709 ± 24306
P7	69722 ± 3495	56629 ± 1681
P14	46070 ± 6704	31976 ± 311
P21	40834 ± 2893	20675 ± 7862
P45	40643 ± 3247	Not estimated
P60	Not estimated	17889 ± 4906
P90	Not estimated	13332 ± 3227
P120	Not estimated	17227 ± 5105
P180	49656 ± 6021	18560 ± 4723

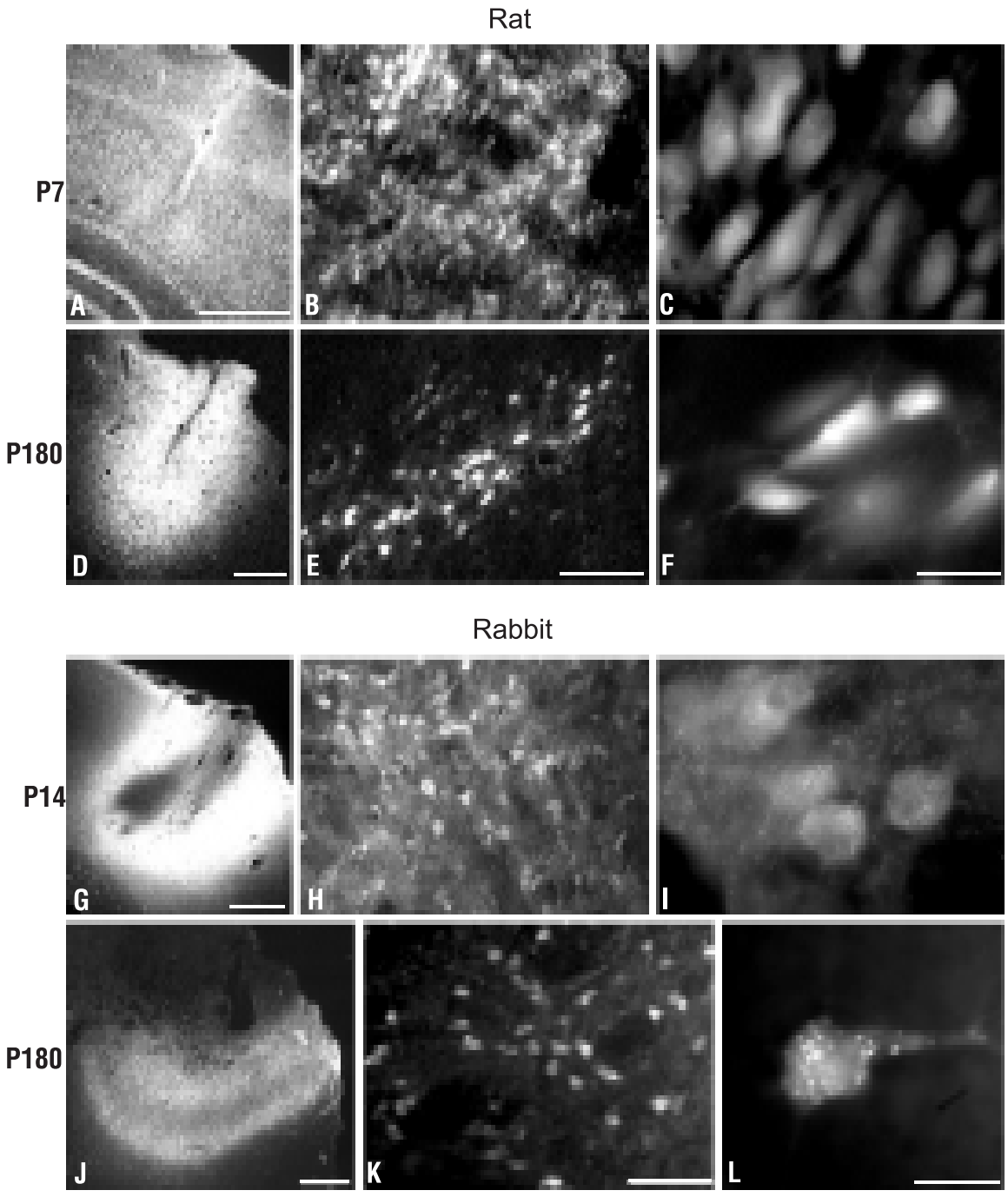

Figure 1. Postnatal changes of the distribution of retrogradely labeled neurons of VPL after injection of the fluorescent tracer into the somatosensory cortex in the two studied species in representative age groups. The site of the tracer (FG) injection (**A**, **D**, **G**, **J**). The projection zone of retrogradely labeled neurons in VPL (**B**, **E**, **H**, **K**). Morphology of single relay neurons of VPL (**C**, **F**, **I**, **L**). Note the significant decrease in the density package of neurons in adult in comparison with the younger age group. Scale bars: A, D, G, J — 500 μm; B, E, H, K — 100 μm; C, F, I, L — 20 μm.

cation of the somatosensory cortex, imprinting the blueprint of the sensory loop [1]. In both studied species a strike of multimodal sensory impulses and their later continuous flow result in the growth acceleration and the network modeling during the first two weeks of postnatal life. These impulses "sculpture" the somatosensory cortex in the process of stimulus strength adjustment to the receiving abilities of the cortex independent of the complexity of intrinsic organization of VPL.

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