Transcription of Rat Cortical Neurons after Homo- and Heterotopic Transplantation at Different Stages of Postnatal Ontogenesis

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UDC 519.881-95-923.4:612.018-393

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 117, № 6, pp. 667-669, June, 1994
Original article submitted January 29, 1993

The template activity of nuclear chromatin of pyramids and stellate neurons of rat sensorimotor cortex during postnatal differentiation is studied in intact brains and after transplantation. In none of the examined periods after homotopic transplantation does the mean level of template activity of the extranucleolar chromatin in different populations of neurons reach the maximum level observed in normal cells. By contrast, at almost at all times after heterotopic transplantation the mean level of transcription is significantly higher than in the control.

Key Words: *chromatin; transplantation; transcription; neuron; sensorimotor cortex*

The transplantation of embryonal nervous tissue in the brain of humans and animals has become an increasingly important research and clinical tool. A large body of experimental evidence has been accumulated on the development and function of nerve cells in a graft [2]. However, numerous aspects of this problem have not been investigated. The use of modern methods is vital for the evaluation of the major parameters of neuronal vitality and their specific features after transplantation.

Our aim was to study the transcriptional activity of chromatin in the neurons of the rat sensorimotor cortex (SMC) after homo- and heterotopic transplantation in different periods of postnatal ontogenesis corresponding to the differentiated and nondifferentiated states.

MATERIALS AND METHODS

Large and medium pyramidal and stellate neurons of the 3rd, 5th, and 4th layers of SMC were studied on days 14, 45, and 90 of postnatal ontogenesis in intact outbred albino rats and after transplantation in the SMC of the cerebral hemispheres or in the cerebellar cortex. Brain cortex of 19-day-old embryos served as grafts. Transplantation was performed by the standard method. The recipient rats were sacrificed 17, 48, and 93 days after the operation.

The transcriptional activity of chromatin was evaluated using the radioautography technique, which reveals the activity of endogenous RNA polymerases in fixed cells on histological preparations [3]. Sections (8 μ thick) of the brain cortex or cerebellum with grafted tissue were cut on a histocryotome at -20°C, air dried, and fixed with an ethanol-acetone mixture (1:1) (4°C, 5 min). Then the sections were incubated at 37°C for 30 min with 0.02 ml of a mixture containing (in mM): Tris-HCl (pH 7.9) 100, sucrose 150, ammonium sulfate 80, 2-mercaptoethanol 12, ^{3}H-UTP 0.02, unlabeled triphosphates 0.6 each; $MgCl_2$ 8, and $MnCl_2$ 2. The reaction was stopped by a thorough washing of the preparations in distilled water, which was followed by a 30-min postfixation with ethanolacetic acid (3:1). Unincorporated phosphates were removed by treatment with 5% trichloroacetic acid (4°C, 15 min); the sections

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0007-4888/94/0006-0676$12.50

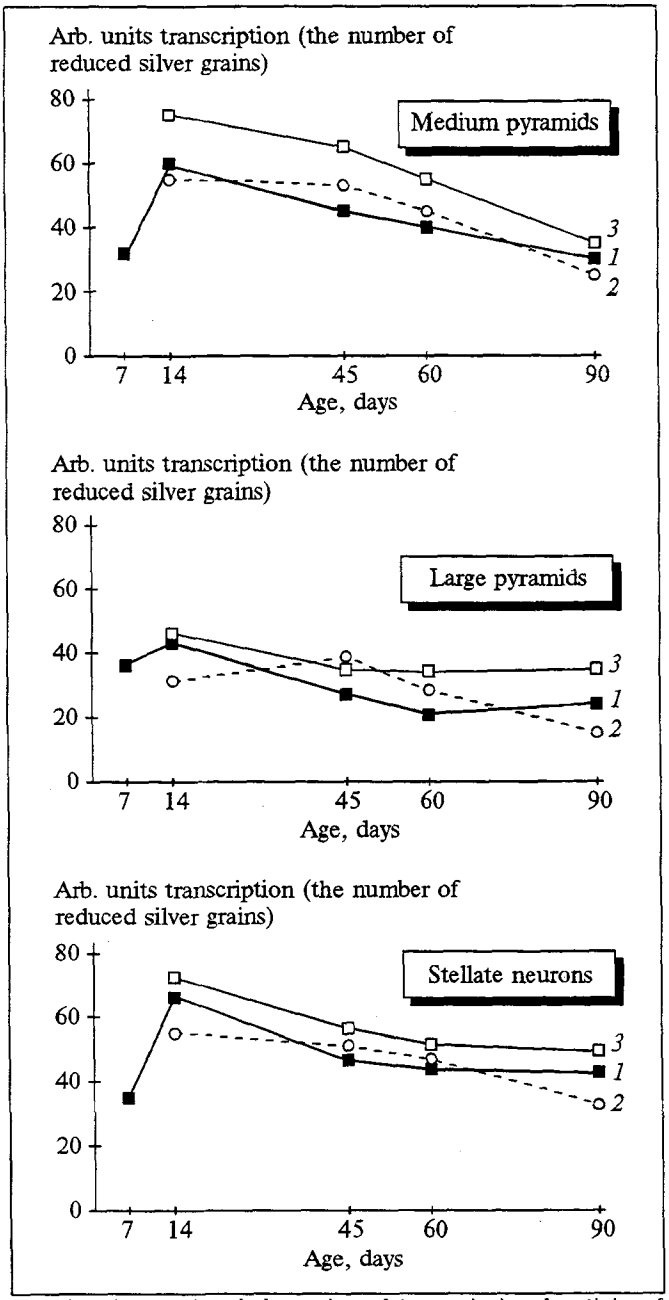

Fig. 1. Age-related dynamics of transcriptional activiy of nucleoplasmic chromatin in rat SMC neurons in the norm (*1*) and after homo- (*2*) and heterotopic (*3*) transplantation.

were washed for 30-60 min under running water, dried, covered with type M emulsion, and exposed for 10 days. The template activity of the nucleus was evaluated by the number of reduced silver grains counted under a light microscope. The reliability of differences in the template activity was determined using the Wilcoxon test.

RESULTS

After homotopic transplantation the changes in the mean levels of transcriptional activity of the extranucleolar sites is essentially similar for all the types of neuronal populations studied, namely, on days 14 and 90 of postnatal ontogenesis this parameter was lower and on day 45 it was higher than in the control (Fig. 1, *b*). Quantitatively speaking, the deviations from the control were small (not more than 20%).

On days 14 and 90 of postnatal ontogenesis, changes in the transcriptional activity of intranucleolar chromatin of neurons in the homograft coincided with those occurring in the extranucleolar sites; on day 45 they were different in the different types of neurons: the activity was increased in stellate neurons, slightly decreased in medium pyra-

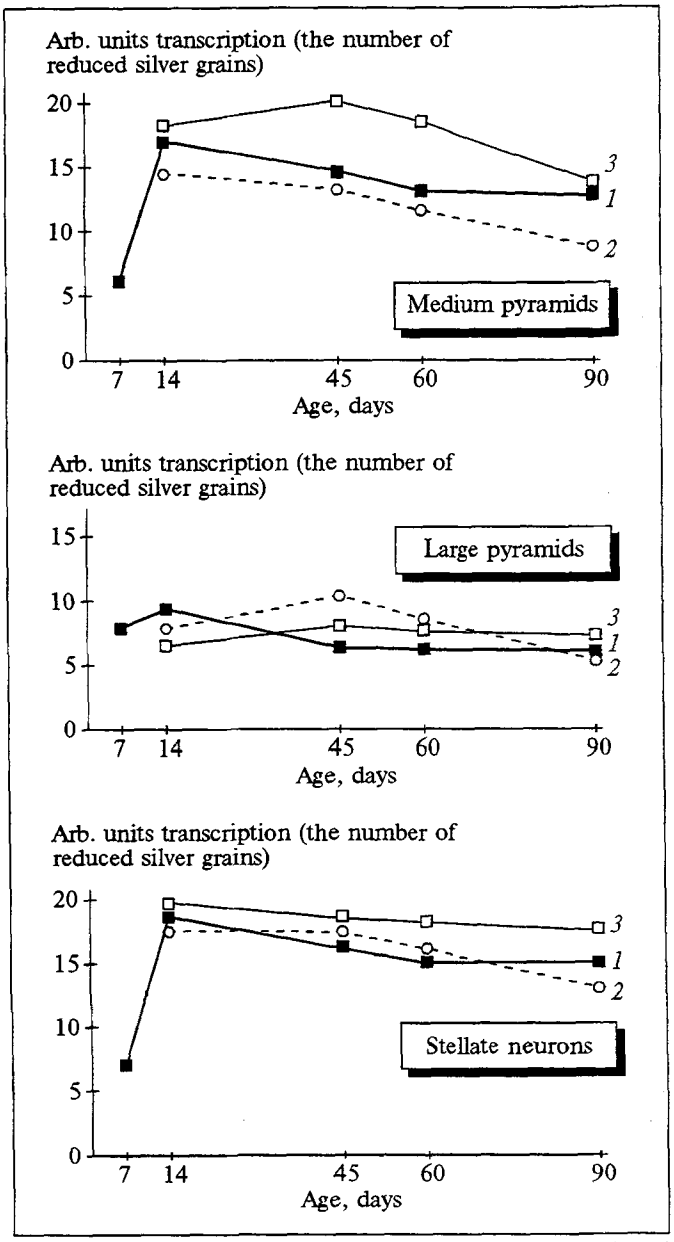

Fig. 2. Age-related dynamics of transcriptional activity of nucleolar chromatin in rat brain SMC neurons in the norm (*1*) and after homo- (*2*) and heterotopic (*3*) transplantation.

mids, and was the same as in the control in large pyramidal cells (Fig. 2, *b*).

On day 45 after heterotopic transplantation extranucleolar transcription on all types of neurons studied was increased compared with the control, as in the case of homotransplantation. The same held true for nuclear chromatin. On days 14 and 90 a tendency toward an increase in transcriptional activity was observed; however, it was different for the different types of neurons (Fig. 1, *c*): the activity in stellate neurons on day 90 and in medium pyramids on day 14 was much higher than in the control and did not exceed 20% in all the other cases. The tendency toward an increase in nucleolar transcriptional activity occurred in different neurons in a heterograft, with the exception of stellate neurons on day 14, which displayed a slight but statistically significant decrease in template activity (Fig. 2, *c*).

The changes in the mean values of transcriptional activity in the studied neuronal populations in grafts are due to a different (compared with the control) percentage ratio of weakly, moderately, and strongly labeled neurons and even to the emergence of very strongly labeled neurons, which are not normal at this age.

The shifts in the transcriptional activity of neurons in transplants are reflected in its age-related dynamics, the nature of the changes in the dynamics being determined more by the type of graft than by the type of neuron.

Normally the transcriptional activity of extranucleolar chromatin in cortical neurons increases during the period between days 7 and 14, as was demonstrated previously [1], and decreases during the period between days 14 and 45. A decrease also occurs between days 45 and 90; however the rate of this decrease is much lower (Figs. 1, *a* and 2, *a*).

After homotopic transplantation extranucleolar transcription in all the examined types of neurons on day 14 was lower than in the control, while the values on days 14 and 45 practically do not differ from the norm (Fig. 1, *b*). The value decreases after day 45 and is somewhat lower than the control on day 90.

After heterotopic transplantation the curve reflecting the age dynamics of transcriptional changes is similar to the control curve, demonstrating a decrease on day 14; at the 14th-45th day interval the curve is almost parallel to the control curve but lies higher and to the right of it, indicating the lagging of the age-related transcriptional reduction behind the norm in this period of postnatal ontogenesis. For stellate neurons this lag is retained at least to the 90th day, while for pyramids the template activity is consistent with the norm (Fig. 1, *c*).

Under the conditions of transplantation the dynamics of the mean values of nucleolar activity changes similarly in almost all cases, although, on the whole, these changes are less pronounced and not as demonstrative as in the case of extranucleolar transcription.

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