Effects of an Imprinting Procedure on Cell Proliferation in the Chick Brain

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We report here studies on the effects of an imprinting procedure on cell proliferation in neonatal chicks in brain structures known to undergo plastic changes in imprinting. Proliferating cells were detected immunohistochemically on brain sections by incorporation of pre-training doses of 5-bromodeoxyuridine (BrdU) into DNA; numbers of new cells were counted in the intermediate medial mesopallium, the intermediate arcopallium, the medial part of the mesopallium and the nidopallium, the dorsocaudal nidopallium, the hippocampus, and the parahippocampal region 24 h and seven days after training. The intermediate medial mesopallium showed an increase in the number of BrdU-positive cells 24 h after training. However, at seven days post-training, the number of BrdU-containing cells decreased in the medial nidopallium and mesopallium, in the dorsocaudal nidopallium, and the right intermediate medial mesopallium. Thus, the imprinting procedure had differently directed transient and long-term influences on the genesis of new cells in the chick brain, inducing the appearance of a large number of cells in the parenchyma of the brain one day after training and decreases in the numbers of cells at later time points. This double effect may be associated with the fact that the imprinting procedure simultaneously initiates two brain processes involving the control of cell proliferation – one related to maturation of a species-specific functional system for tracking individuals of the same species and one related to remembering the characteristics of the actual parent.

KEY WORDS: imprinting, training, cell proliferation, BrdU.

Neurogenesis and its interaction with the formation of mother-following responses in neonatal chicks is of particular interest. Imprinting is a special type of learning, combining innate behavior and the acquisition of individual experience [8, 17]. Imprinting is almost irreversible and can only occur during the first 1–3 days after hatching [8]. It is known that during the first days of life, new cells are produced in the chick brain very actively [12]. If cell proliferation does in fact have some involvement in the process of memory formation on imprinting, then impression of the imprint object might be expected to be reflected in the quantity of newly formed cells in brain structures, in turn reflecting their involvement in the formation of the preference.

The aim of the present work was to verify this hypothesis by studying the influences of imprinting on cell proliferation in the brains of neonatal chicks by administering the proliferation marker 5-bromodeoxyuridine (BrdU).
METHODS

Studies were performed on Lomon Brown chicks of both genders. Experimental protocols were approved by the Bioethics Committee of the P. K. Anokhin Research Institute of Normal Physiology, Russian Academy of Medical Sciences. Chick embryos were obtained from a poultry producer at age 13–15 days and were kept in a light- and sound-proofed incubator at 37.5°C to hatching. Chicks were then placed in a specially equipped thermostatted incubator, where they were kept for one day after hatching in the dark at 33°C.

At age one day all chicks received i.p. BrdU solution at a dose of 100 mg/kg; 30 min after injections, control chicks were placed in home cages of size 20 × 20 × 25 cm, in which they were subsequently kept in pairs with unlimited access to water and food. Chicks of the two experimental groups, 30 min after BrdU injections, were imprinted for 1 h and were then placed in home cages. At 24 h post-injection, chicks of one control group and one experimental group were decapitated and brains were removed. Chicks of the other two groups were decapitated 7 days after BrdU injections.

The imprinting model was based on that of B. McCabe et al. [21]. Imprinting was performed in a running wheel to an illuminated rotating stuffed chick for 60 min in the dark at 28 ± 1°C. The total activity of the chicks during the procedure was increased by playing recordings of maternal chick calls. Previous studies have demonstrated that this imprinting procedure leads to a marked preference for the imprinted object [20]. Additional experiments were also performed to verify that injection of 100 mg/kg of 5-bromodeoxyuridine did not prevent memory formation on imprinting. This involved the use of two additional groups of chicks: one control group (n = 17), given 100 mg/kg BrdU and placed in home boxes 30 min later, and one experimental group (n = 13), imprinted for 1 h 30 min after injection of 100 mg/kg of BrdU. Animals of both groups were tested for preference for the imprinted object (the stuffed chick) 3 and 24 h after training.

Chicks were tested by simultaneous presentation of the object used for imprinting (the stuffed chick) and a new object (a red cube) for 10 min. The level of preference was measured as the coefficient of preference, i.e., the ratio of the number of turns of the wheel towards the stuffed chick to the total number of turns.

For immunohistochemical reactions for BrdU, chicks were decapitated and brains were removed and frozen in liquid nitrogen vapor. The immunohistochemical reaction was performed in plates (Thermo Shandon, Coverplate) on cryostat sections of thickness 20 µm. Sections were prefixed in 4% paraformaldehyde and kept in 2N HCl solution to denature DNA. Immunohistochemical reactions were performed using monoclonal antibodies to BrdU (mouse anti-BrdU, Chemicon, diluted 1:200, incubated for 12–18 h) followed by washing and incubation with secondary antibodies (horse anti-mouse IgG, Vector, Elite ABC Kit, diluted 1:200); sections were then washed and plates were loaded with AB complex (avidin + biotin 1:1, Elite ABC Kit, antimouse IgG, Vector, diluted 1:100) and incubated for 1 h. After incubation, sections were washed, removed from their containers, and stained with diaminobenzidine solution. Slides were then passed through sequential alcohols of increasing concentrations and embedded under cover slips.

BrdU-positive cells in brain structures were counted using the AnalySIS 2.5 image analysis system; data were analyzed statistically in Statistica 6.0 running the Mann–Whitney test for comparison of experimental groups and the Wilcoxon test for analysis of interhemisphere asymmetry. The densities of BrdU-labeled cells in each structure were calculated as the ratio of the number of BrdU-positive cells to the area occupied by the structure on the section. The locations of structures containing BrdU-positive cells were determined on parallel sections stained by the Nissl method using a stereotaxic atlas of the chick brain (Kuenzal and Mason, 1988). The required structures were outlined by hand in the AnalySIS program.

Quantitative analysis of the densities of BrdU-labeled cells was performed in structures critical for visual imprinting, i.e., the intermediate medial mesopallium (IMM) and the intermediate arcopallium (AI), in areas needed for auditory imprinting, i.e., the dorsocaudal nidopallium (Ndc) and medial part of the mesopallium and nidopallium (MN), as shown in the figure.