ACTA NEUROBIOLOGIAE EXPERIMENTALIS

Acta Neurobiol. Exp. 1998, 58: 23-28

Postnatal development of the rat striatum - a study using *in situ* DNA end labeling technique

Beata Maciejewska, Małgorzata Lipowska, Przemysław Kowiański, Beata Domaradzka-Pytel and **Janusz Moryś**

Department of Anatomy and Neurobiology, Medical University of Gdańsk, 1 Dębinki St., 80-211 Gdańsk, Poland, Email: jmorys @ amedec. amg. gda. pl

Abstract. We have examined the development of rat striatum for evidence of cells dying in the process of physiological cell death. In present study we have indicated apoptotic cells in sections stained with cresyl violet (cell death characterized by pyknosis) or with DNA end labeling assay (TUNEL method). Our results demonstrated that cell loss during maturation of the rat striatum had the characteristics of apoptosis rather than necrosis. The greatest number of TUNEL - positive and pyknotic cells in the striatum were found during the first postnatal days; after 7th day of postnatal life a rapid decrease of its number was observed. After the second postnatal week no TUNEL-positive cells were observed in the striatum. Our analysis suggests that apoptotic cell death occurring during the development of striatal neuronal population takes place during the first week of postnatal life.

Key words: striatum, development, apoptosis, rat, TUNEL

INTRODUCTION

In the rat, striatum is a large, telencephalic structure composed of two main parts: dorsal and ventral (Heimer et al. 1995). The ventral striatum should be regarded as a ventral continuation of the dorsal striatum in the sense that in cresyl violet staining no clear boundary can be drawn between these two regions (Heimer et al. 1995).

The striatum can be divided into two compartments, the matrix and striosom or patch. In coronal sections the striosom appears as irregularly shaped islands that form three-dimensional labyrinths within the matrix. In spite of considerable effort, the functions of these two compartments of the striatum are still far from clear (Heimer et al. 1995).

During the development a massive cell death occurs in many regions of the brain (Clarke 1985, Oppenheim 1991, Ferrer et al. 1992). This is a normal feature of neurogenesis (Oppenheim 1985). The characteristic evidence of apoptotic cell death includes cell shrinkage, condensation of nuclear chromatin and appearance of apoptotic bodies (Ferrer et al. 1992, Ferrer et al. 1994). Sections stained with cresyl violet reveal pyknotic neurons and the absence of inflammatory reaction (Clarke 1990, Holman et al. 1996). At the biochemical level, apoptosis is associated with DNA fragmentation that can be detected *in situ* by TUNEL method [TdT mediated dUTP nick end labeling of fragmented DNA] (Gavrieli et al. 1992, Gorczyca et al. 1993).

The semiquantitative analysis and distribution of apoptotic cells during maturation of the rat striatum was the main subject of our present study.

METHODS

The material consisted of 38 rat brains of different postnatal age: P1, P3, P4, P5, P7, P8, P9, P10, P14, P18, and P21. In each group, three or four animals were studied.

Care and treatment of the animals were in accordance with the guidelines for laboratory animals established by the National Institute of Health as well as by the local ethical committee. All animals were anesthetized by intraperitoneal injection of lethal dose of Nembutal (80 mg/kg of body weight). The brains were fixed by transcardiac perfusion with the 4% paraformaldehyde solution in phosphate buffer; then removed from the skull, weighted and fixed in the same solution for maximum 1 week. After fixation the brains were embedded in paraffin, cut coronally into 10-μm-thick serial sections. Every 10^{th} section containing striatum was used for study. Each step was precisely timed; the embedding process lasted 7 days. The sections were stained either with cresyl violet or processed for in situ labeling.

In situ labeling of nuclear DNA fragmentation (TUNEL)

After deparafinization, sections were pre-treated with proteinase K (25 μg/ml, Sigma) for 15 min, followed by 3% H_2O_2 for 10 min and preincubated in TdT buffer (30 mM Trizma base, 140 mM sodium cacodylate pH 7.2, 1 mM cobalt chloride). Then, sections were incubated in the same buffer containing TdT (0.05 U/μl, Boehringer Mannheim) and biotin-16-dUTP (0.01 nmol/μl, Boehringer Mannheim) in humid atmosphere and 37°C for 60 min. The reaction was terminated by washing the slides in 300 mM NaCl and 30 mM sodium citrate buffer for 15 min. Then sections were treated by 2% BSA (10 min), incubated with avidin-biotin-peroxidase complex (ABC kit, Vectastain, Vector) for 60 min, followed by diaminobenzidine/H_2O_2 and counterstained with cresyl violet. Positive controls included treatment of the sections with DNase.

Cell counts and mapping of apoptotic cells

The identification of neurons was performed using the standardized cytological criteria based on the presence of specific chromatin patterns and emerging dendrites (Rabinowicz et al. 1996). Cresyl violet stained cells with apoptotic morphology (pyknotic cells) and TUNEL-positive nuclei were localized using light microscope. The total number of apoptotic cells present in each section was counted in the area of striatum delineated as in Fig. 2. To determine the distribution of apoptotic cells, their localization was mapped. The majority of apoptotic cell death occurred midway between rostral and caudal part of the striatum. Three representative sections from this area were plotted onto an outline of the striatum (Fig. 2).

RESULTS

On the first day of postnatal life the rat striatum was well developed - two main striatal nuclei: the caudate-putamen (a larger, dorsal part of the striatum) and the nucleus accumbens (a smaller, ventral part) were clearly visible. At this stage the greatest number of TUNEL-

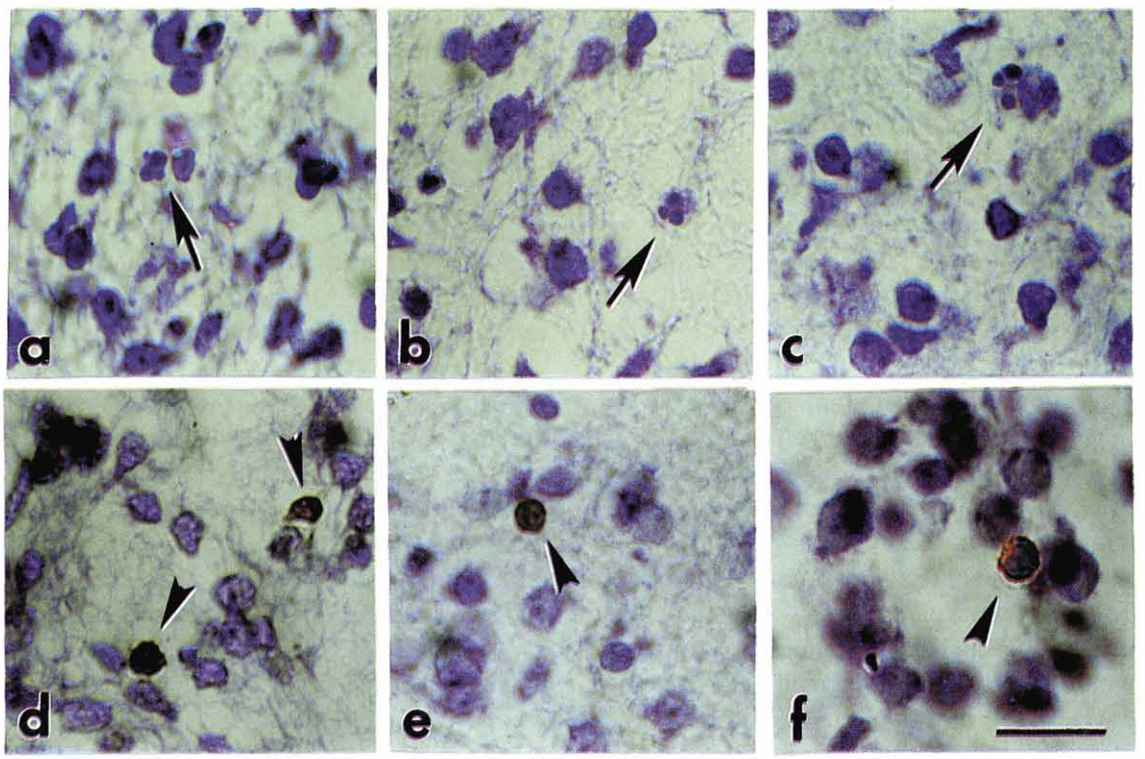

Fig. 1. Neurons in the striatum at various stages of postnatal life. a, *b, c*, cresyl violet stain; arrows indicate cells with apoptotic morphology characterized by extreme chromatin condensation and fragmented nuclei with apoptotic bodies at age: P1 *(a)*, P5 *(b)* and P7 *(c)*. *d, e, f*, cells bearing fragmented DNA (arrowhead), revealed by *in situ* DNA fragmentation staining at stages: P1 *(d)*, P4 *(e)* and P7 *(f)*; P, postnatal days; bar = 14 μm.

positive cells were observed (range: 25-46 per section); their morphology is represented on Fig. 1d. However, majority of striatal cells were darkly stained with cresyl violet, few pyknotic cells were also found among them (Fig. 1a). These cells were distributed quite evenly in the whole striatum.

The high level of TUNEL positive striatal cells were maintained during the first postnatal week (range: 22-40

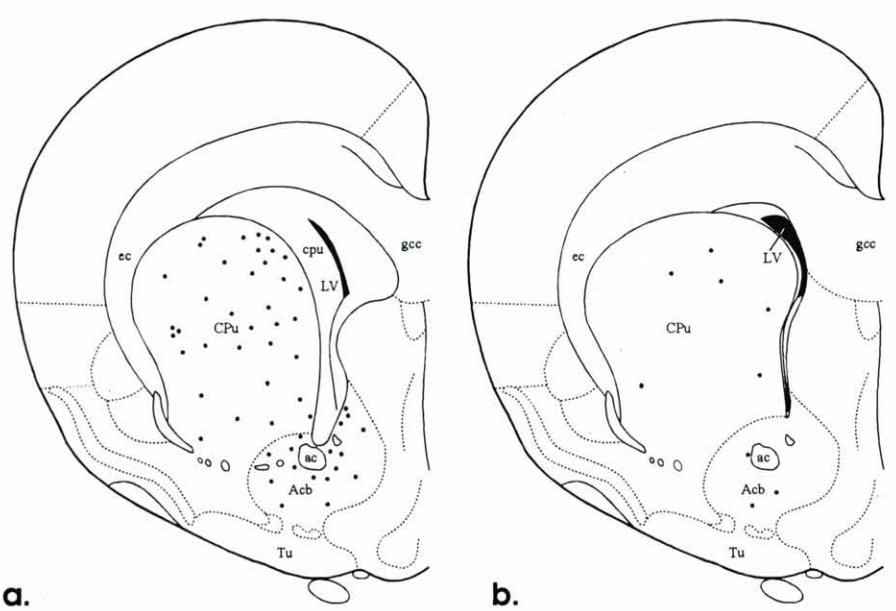

Fig. 2. An anatomical diagram of rat brain showing the location of TUNEL-positive cells in the caudate-putamen and nucleus accumbens on day P1 (a) and P10 (b). Sections from mid striatum. *ac*-anterior commissure, *Acb*-nucleus accumbens, *CPu*-caudate putamen, *cpu*-caudate putamen neuroepithelium, *gcc*-genu corpus callosum, *LV*-lateral ventricle, *ec*-external capsule, *Tu*-olfactory tubercle.

per section at P3, 18-28 per section at stages P4 - P7); their morphology is represented on Fig.1e,f. After this period (from 7th day of postnatal life) the number of TUNEL-positive cells rapidly decreased. In cresyl violet staining, pyknotic cells were observed in the whole striatum but their number was smaller than that at stage P1. Morphology of pyknotic cells varied from those with dense, structureless nuclei and cytoplasm to neurons having dense fragmented, nuclei surrounded by a rim of clear cytoplasm (Fig. 1a,b and c).

At stages P14, P18 and P21 no TUNEL-positive cells were observed in the whole striatum.

The apoptotic cells were found in all areas of the striatum; sporadically they tended to cluster in-group of two to three cells. The distribution of dying cells throughout the striatum was not completely homogeneous (Fig. 2). In the caudate-putamen, relatively more apoptotic cells appeared near the dorsal part of the striatal neuroepithelium, while in the nucleus accumbens - around the anterior commissure and the subventricular zone surrounding the inferior horn of the lateral ventricle (Fig. 2). In the rostro-caudal direction, the majority of the apoptotic cells were found in the central portion of the striatum. The TUNEL-positive and pyknotic cells were observed mainly (but not only) in the matrix compartment of the striatum.

The density and distribution of apoptotic cells were similar in different animals of the same age.

At each stage (mainly during the first week) a few cells with pyknotic morphology but without DNA fragmentation (TUNEL-negative) were observed.

During the first postnatal week, the mitotic figures were identified in both caudate-putamen and nucleus accumbens. Nuclei of mitotic cells were seen in all stages of proliferation but particularly in the metaphase and anaphase (Fig. 3). The mitotic figures were observed mainly in the patch compartment of the striatum.

DISCUSSION

We have found that cell elimination during maturation of the caudate-putamen and nucleus accumbens in rat may be regarded as a result of apoptosis rather than necrosis. Dying cell nuclei showed chromatin condensation (pyknosis) but no swelling, dispersion, or flocculation of chromosomal material; these processes generally occur in nuclei of necrotic cells (Clarke 1990).

In the present study we identified apoptotic cells in sections stained with cresyl violet (cell death characterized by pyknosis; Holman et al. 1996) or with DNA end labeling assay (TUNEL method). The greatest number of apoptotic cells in the striatum was found during the first postnatal days; after 7th day of postnatal life a rapid decrease of their number was observed.

According to our results the highest number of apoptotic cells occurred, like in the globus pallidus (Waters et al. 1994), in the central portion of the striatum. In frontal sections they were found in all areas of the striatum, where they were scattered, rather than gathered as in necrotic cell death; although, sometimes apoptotic cells tended to cluster in groups of two to three cells (Fig. 2). The gathering of cells can be explained in two ways: the programmed death of sister cells born at the same time or due to the local influence of external factors such as excitotoxins or growth factor acting on cells of the same genotype (Waters et al. 1994).

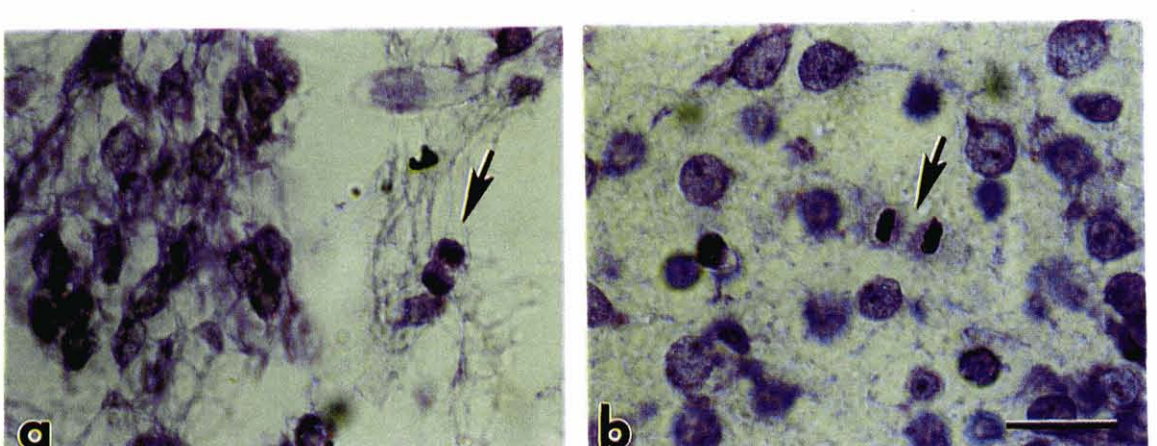

Fig. 3. Mitotic figures *(arrows)* in the striatum at postnatal stages: P4 *(a)* and P7 *(b)*. P, postnatal days. Cresyl violet; bar = 14 μm.

The ontogeny of the apoptotic cells observed in the neonatal striatum is uncertain; however, our results suggest that most of dying cells were of neuronal origin. This assumption is based primarily on the chromatin condensation and neuronal morphology in the light microscope. However, the possibility that some of the apoptotic cells are of glial origin can not be excluded.

The presence of some cells with pyknotic morphology but without DNA fragmentation (TUNEL-negative) could mean that not all cells died due to apoptosis in the neonatal striatum. Appearance of cells that exhibited condensed chromatin without DNA fragmentation was observed also in other brain structures (cerebellum - Wood et al. 1993 and globus pallidus - Waters et al. 1994).

The globus pallidus (Waters et al. 1994), substantia nigra (Janec and Burke 1993) and ventrobasal complex of thalamus (Waite et al. 1992) exhibit peak of cell death at the same time as in the developing striatum. These structures are involved in the control of movement. The connections between globus pallidus, substantia nigra, ventrobasal complex of thalamus and striatum are formed mainly during prenatal life and appearance of apoptotic cell death in this structures during first postnatal days may be a consequence of elimination of neurons that have failed to make appropriate connections. Because apoptotic cell death in developing cerebral cortex and subcortical white matter in the rat appears mainly between P3 and P7 (Ferrer et al. 1994) it is possible that the death of striatal cells taking place at the end of the first postnatal week is caused by elimination of neurons that form inappropriate cortical connections. We can not exclude the possibility that some of dying striatal cells are neurons which have failed to migrate to an appropriate site from ventricular zone.

The presence of relatively large number of dying cells near the boundary of the ventricular neuroepithelial zone can suggest that these cells may form at this place a temporary structure gradually that become redundant and can be eliminated (like transiently appearing cortical sub-plate). Such hypothesis may explain loss of neurons in rat globus pallidus (Waters et al. 1994).

Although some mitotic figures were present both in caudate-putamen and nucleus accumbens we do not suggest that neurogenesis is taking place in these nuclei. Cell proliferation in brain tissues undergoing apoptosis was observed early by Johnson and Bottjer (1994) and Holman et al. (1996). According to Bowen and Bowen (1990) the dividing cells are phagocytes that remove cell debris. It is possible that dividing microglia are activated by some unknown stimuli to phagocyte cell debris from neurons that have died and began the process of fragmentation (Thomas 1992).

The present results indicate that establishment of the final cell population in the rat striatum takes place during the first week of postnatal life and is associated with apoptotic death of neurons.

ACKNOWLEDGEMENTS

The authors wish to thank Mrs Aleksandra Arceusz M.A., for copy-editing the manuscript. This research was supported by funds from Committee of Scientific Research, Grant No ST-11.

REFERENCES

Bowen J.D., Bowen S.M. (1990) Programmed cell death in tumors and tissues. Chapman and Hall, London.

Clarke G.H. (1990) Developmental cell death: morphological diversity and multiple mechanisms. Anat. Embryol. 181: 195-213.

Clarke P.G.H. (1985) Neuronal death in the development of the vertebrate nervous system. TINS 8: 345-349.

Ferrer I., Soriano E., del Rio J.A., Alcantara S., Auladell C. (1992) Cell death and removal in the cerebral cortex during development. Prog. Neurobiol. 39: 1-43.

Ferrer I., Tortosa A., Blanco R., Martín F., Serrano T., Planas A., Macaya A. (1994) Naturally occurring cell death in the developing cerebral cortex of the rat. Evidence of apoptosis-associated internucleosomal DNA fragmentation. Neurosci. Lett. 182: 77-79.

Gavrieli Y., Sherman Y., and Ben-Sasson S.A. (1992) Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentation. J. Cell. Biol. 119: 493-501.

Gorczyca W., Gong J., Darzynkiewicz Z. (1993) Detection of DNA strand breaks in individual apoptotic cells by the in situ terminal deoxynucleotidyl transferase and nick translation assays. Cancer Res. 53: 1945-1951.

Heimer L., Zahm D.S., Alheid G.F. (1995) Basal ganglia. In: The rat nervous system (Ed. G. Paxinos). Academic Press, New York, p. 579-628.

Holman S.D., Collado P., Skepper J.N., Rice A. (1996) Postnatal development of a sexually dimorphic, hypothalamic nucleus in gerbils: a stereological study of neuronal number and apoptosis. J Comp. Neurol. 376: 315-325.

Janec E., Burke R.E. (1993) Naturally occurring cell death during postnatal development of the substantia nigra pars compacta or rat. Mol. Cell. Neurosci. 4: 30-35.

Johnson F., Bottjer S. W. (1994) Afferent influences on cell death and birth during development of a cortical nucleus necessary for learned vocal behavior in zebra finches. Development 120: 13-24.

Oppenheim R.W. (1985) Naturally occurring cell death during neural development. TINS 11: 487-493.

Oppenheim R.W. (1991) Cell death during development of the nervous system. Ann. Rev. Neurosci. 14: 453-501.

Rabinowicz T., De Courten-Myers G. M., Petetot J. M.C., Xi G.H., De los Reyes E. (1996) Human cortex development: estimates of neuronal numbers indicate major loss late during gestation. J. Neuropathol. Exp. Neurol. 55: 320-328.

Thomas W. E. (1992) Brain macrophages: evolution of microglia and their functions. Brain Res. Rev. 17: 61-74.

Waite P.M.E., Lixin L., Ashwell K.W.S. (1992) Development and lesion induced cell death in the rat ventrobasal complex. Neuroreport 3: 485-488.

Waters C.M., Moser W., Walkinshaw G., Mitchell I.J. (1994) Death of neurons in the neonatal rodent and primate globus pallidus occurs by a mechanism of apoptosis. Neuroscience 63: 881-894.

Wood K.A., Dipasquale B., Youle R.Q.J. (1993) In situ labeling of granule cells for apoptosis-associated DNA fragmentation reveals different mechanisms of cell loss in developing cerebellum. Neuron 11: 621-632.

Received 21 October 1997, accepted 15 January 1998