Доклади на Българската академия на науките
Comptes rendus de l'Académie bulgare des Sciences

Tome 66, No 7, 2013

MEDECINE

Morphologie

PARVALBUMIN IMMUNOREACTIVITY CHANGES DURING THE MATURATION OF THE THALAMIC RETICULAR NUCLEUS OF THE RAT

Lina Malinova, Boycho Landzhov, Anastasia Bozhilova-Pastirova, Lazar Jelev, Dimka Hinova-Palova, Wladimir Ovtscharoff

(*Submitted on March 21, 2013*)

Abstract

The thalamic reticular nucleus is a thin layer of GABAergic cells located between the external medullary lamina and the internal capsule surrounding the rostolateral surface of the thalamus. It has functionally distinct afferent and efferent connections with thalamic nuclei, the neocortex, the basal forebrain and the brainstem. Parvalbumin is a calcium-binding protein, which is regarded to be a marker for GABAergic neurons. The thalamic reticular neurons are GABAergic, and parvalbumin is always colocalized with GABA in these cells. We have demonstrated the parvalbumin immunoreactivity in the thalamic reticular nucleus at different stages of postnatal development of rats, as well at 1-year-old rats. It was established that the maturation of immunoposive patterns varies in different parts of the nucleus. The intensity of immunostaining decreases with age.

Key words: thalamic reticular nucleus, parvalbumin, maturation

Introduction. The thalamic reticular nucleus (TRN) develops from the ventral thalamus [1, 2]. TRN is a thin sheet of neurons surrounding the rostolateral aspect of the thalamus. Located between thalamus and cortex, the TRN occupies a strategic position among the thalamic nuclei. The cytoarchitectonic organization of the TRN has been described by several authors [2–4]. Lubke [5] divided the TRN into dorsal, intermediate and ventral parts. The TRN has been subdivided into specific sectors – motor, limbic, somatosensory, visual and auditory, based on the predominant sensory different modality of the input arriving

via collaterals from the cortex and thalamus [6]. This nucleus receives topographically organized collaterals from both thalamus and cortex and sends similarly organized projections back to thalamus. The inputs to the TRN are excitatory, while the output back to the thalamic relay is inhibitory [3, 6, 7]. The rostral part of TRN has connections with motor and limbic centres [8, 9], whereas the intermediate part is connected with the ventrobasal thalamus and somatosensory cortex [1, 10]. The caudal part of TRN is associated dorsally with the lateral geniculate nucleus and visual cortex, and ventrally with the medial geniculate nucleus and auditory cortex [11, 12].

Parvalbumin is a low-molecular weight calcium-binding protein occurring broadly in various parts of the mammalian nervous system. The protein has the ability to modulate the intracellular calcium signals and changes the mechanism of calcium homeostasis essential for maintenance of the neuronal integrity [3].

Recent studies suggest that TRN may be involved in the neurobiology of schizophrenia, control of absence epilepsy, flexible behaviour, Alzheimer's disease, Parkinson's disease and epilepsy [8, 9, 13].

The aim of the present study was to investigate the distribution and structure of parvalbumin immunoreactive neurons in TRN during postnatal development and in aged rats.

Material and methods. The present study was carried out on 15 Sprague–Dawley rats visualized by means of immunocytochemistry for parvalbumin in the thalamic reticular nucleus at the 20- (P20), 30- (P30) and 60-day-old (P60) and 1-year-old rats. The animals were anaesthetized intraperitoneally with thiopental (40 mg/kg b.w.). Transcardial perfusion with 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.2 was done. The brains were removed from the skulls and postfixed for 1 h in the same fixative, after which they were washed in 0.1 M phosphate buffer, pH 7.2, overnight at 4 °C. Coronal sections were cut on a freezing microtom (Reichert–Jung) at 40 µm prepared for immunocytochemical demonstration of parvalbumin. Free-floating sections were incubated overnight with a mouse monoclonal antibody against parvalbumin (Sigma 1: 1000) diluted in 1% normal serum in 0.01 M PBS and 0.1% Triton X-100. After multiple rinses in PBS, sections were incubated for 90 min in biotinylated horse antimouse IgG (1: 200) diluted in PBS-NHS and 0.2% Triton, rinsed in PBS and incubated in the avidin-biotin peroxidase complex diluted in PBS.

This step was followed by washing in PBS and then in 0.05M Tris-HCl buffer, pH 7.6, which preceded incubation in 0.05% 3,3′-diaminobenzidine tetrahydrochloride (DAB, Sigma) containing 1% H_2O_2 (1:100) for visualization of the reaction. After that, the slices were mounted on the glass slides, dehydrated and coverslipped. The immunohisochemical control, in which the primary antibody was omitted, did not show the positive immunostaining.

Results. The immunostaining for parvalbumin was visible in cell bodies, in the nucleus, as well as in the dendrites (Fig. 1). The parvalbumin immunore-

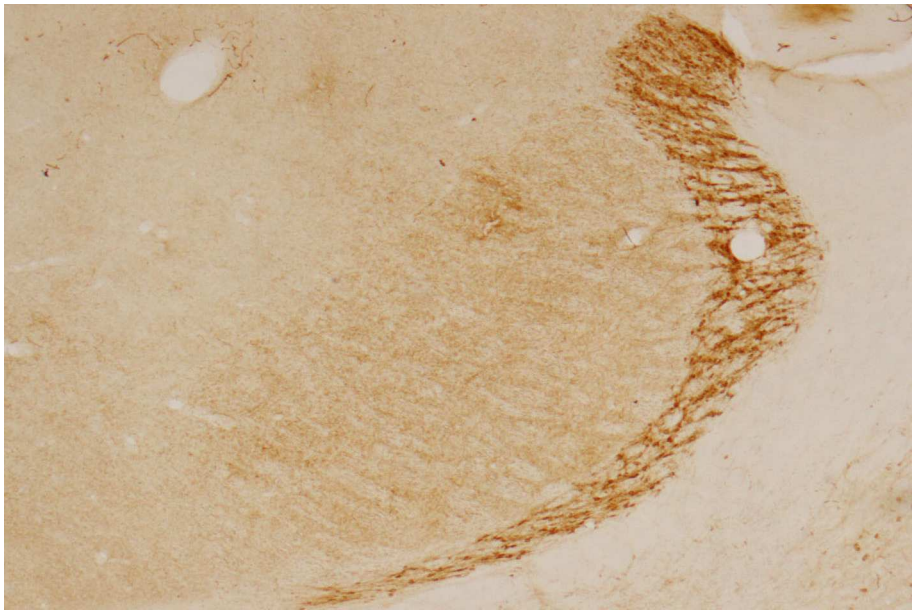

Fig. 1. Parvalbumin immunoreactive cell bodies and dendrites. × 4

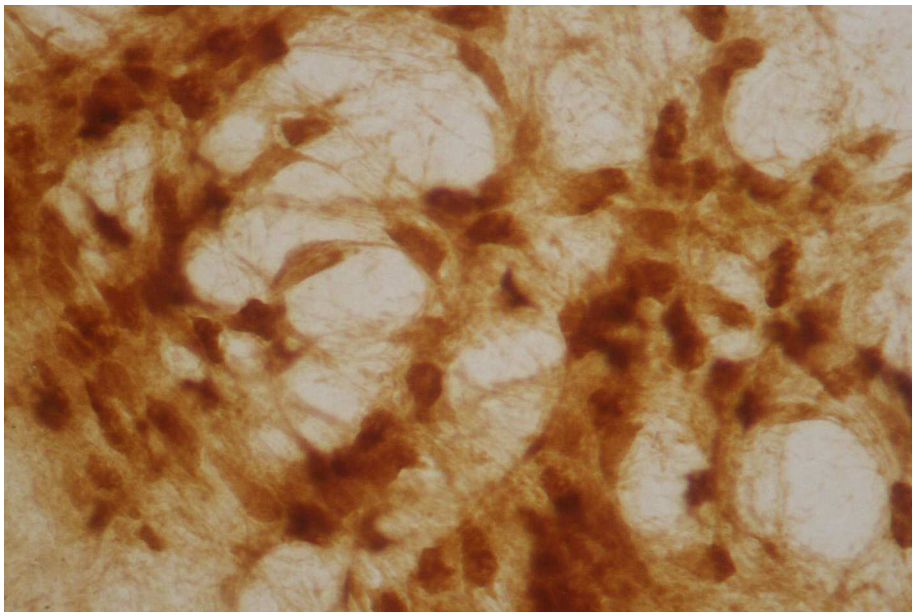

Fig. 2. Fusiform, oval and multipolar in shape neurons in the middle part of the TRN. × 40

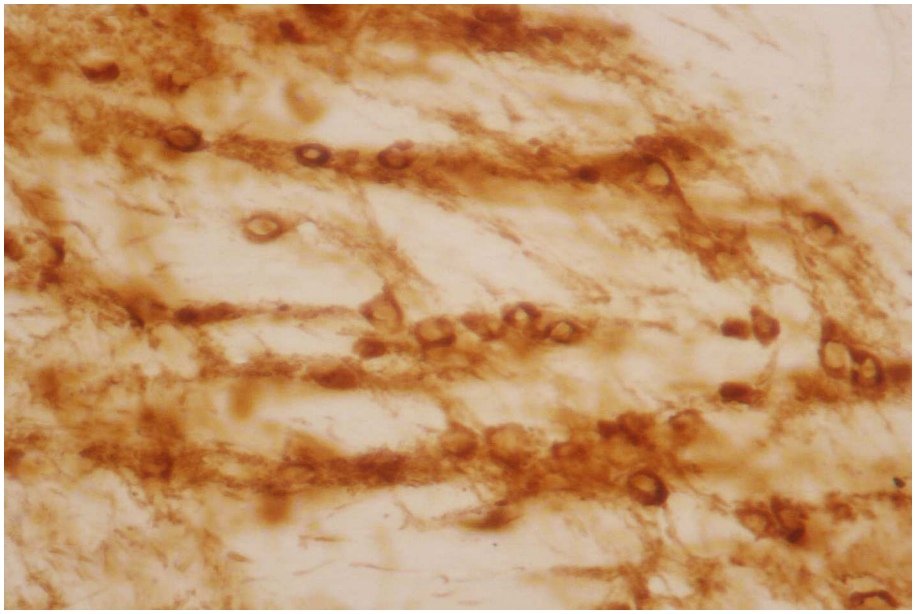

Fig. 3. Oval in shape neurons in the middle part of the TRN. × 40

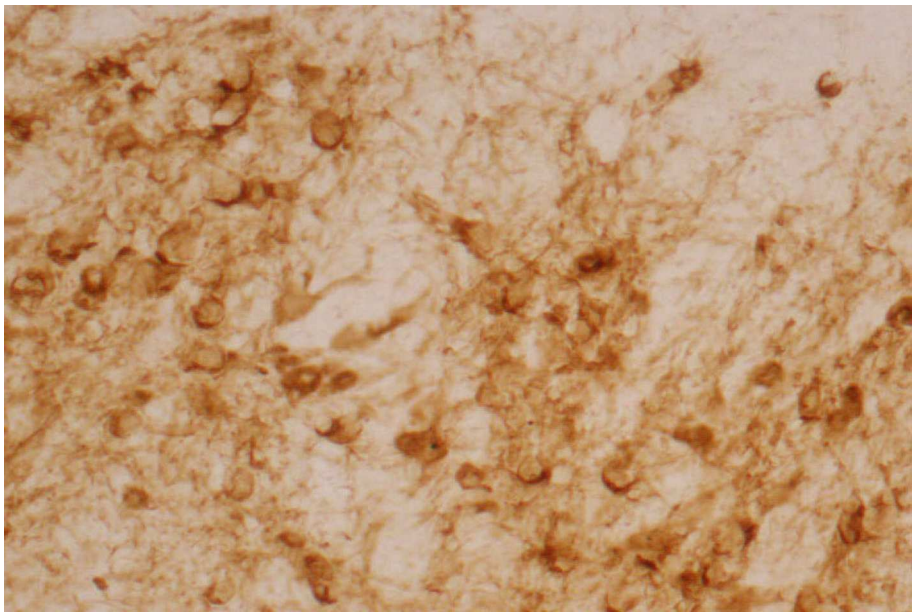

Fig. 4. A lower intensity of the parvalbumin immunoreactivity in the TRN in adult rats. × 40

activity maturation did not occur at the same time in the various parts of the nucleus. At P20 we observed intensive parvalbumin labelling of the neurons and neuropil in some parts of the nucleus, the middle one (Fig. 2). A low number of puncta and parvalbumin positive fibres were present mainly in the visual and auditory areas of the nucleus in its caudal part.

At P30 multipolar neurons with fusiform and oval form were distributed along the rostrocaudal and dorsoventral axis of the nucleus. In the dorsorostral portion, oval neurons were visible (Fig. 3), as well as in the intermediate part, the fusiform neurons were more numerous. The dendrites could be followed in all directions away from the cell body.

At P60 no differences were found in the morphological pattern of parvalbumin reactivity (Fig. 4).

We compared the earliest stages of development with annual rats to establish if there were any changes of parvalbumin immunoreactive neurons. As a result, we did not find any difference in the morphology of the neurons of TRN, but the intensity of the parvalbumin immunoreactivity was lower.

Discussion. The cells of the TRN are described as the earliest neurons that appear in the thalamus anlage. The central part of the nucleus called reticular protuberance is formed earlier (E13), whereas lateral and medial parts located beneath the protuberance generate later (E14). Most of the neurons of the TRN are settled by the 19th embryonic day [14]. PV-immunoreactivity is clearly evident at birth in the cell bodies of the thalamic reticular nucleus [7].

The thalamic reticular nucleus has previously been described as a sheet of homogenous cells that surround most of the rostrolateral surface of the dorsal thalamus, which are GABAergic. It lies medial to the fibres of the internal capsule and lateral to the external medullary lamina of thalamus [1]. The present results show that there is cytoarchitectonic heterogenesis in the reticular thalamic nucleus of rat. These findings correspond to several previous studies in cats and ferrets [3]. PV and GABA were examined as markers of TRN neurons during the postnatal development and in the adult [3, 7, 15, 16]. Parvalbumin is also presented in the GABAergic neurons in different parts of the central nervous system [3, 17, 18], but this colocalization is not permanent, like in the TRN [3].

Calcium binding protein parvalbumin plays an important role in the regulation of neuronal responses, since they are implicated in the buffering and transport of calcium and in the regulation of related enzyme systems [13]. During the individual development of the rat there are two waves of parvalbumin expression. The first wave is detected between postnatal day P0 and P9–11 in the rostolateral surface of the nucleus. The second wave starts from P7–8 to adult. Parvalbumin expression is apparent first in the central, then in the caudal, and finally in the rostral part of the nucleus [19].

The function of TRN is still unknown. However, the direct relation between the morphological features of PV reticular neurons and their neurochemical prop-

erties as well as the concrete function of PV during development remains unclear. For example, the number and size of PV-immunoreactive neurons are significantly decreased in Alzheimer's disease although that decrease is not specific only for it [20]. Probably, the colocalization of parvalbumin and GABA increases the inhibitory effect of the TRN neurons.

REFERENCES

[1] Jones E. G. Proc. Natl. Acad. Sci., **104**, 2007, No 46, 17903–17904.
[2] Clemence A. E., J. Mitrofanis. J. Comp. Neurol., **332**, 1992, No 2, 167–180.
[3] Celio M. R. Neurosci., **35**, 1990, No 2, 375–475.
[4] Amadeo A., B. Ortino, C. Frassoni. Anat. Embriol., **203**, 2001, No 2, 109–119.
[5] Lubke J. J. Comp. Neurol., **329**, 1993, No 4, 458–471.
[6] Pinault D. Brain Res. Rev., **46**, 2004, No 1, 1–31.
[7] Frassoni C., M. Bentivoglio, R. Spreafico, P. Sanches, L. Puelles, A. Fairen. Dev. Brain Res., **58**, 1991, No 2, 243–249.
[8] Barbas E., B. Zikopuolos. Neuroscientist., **13**, 2007, No 5, 532–545.
[9] Gulcebi M. I., S. Ketenci, R. Linke, H. Hacioglu, H. Yanali, J. Veliskova, S. L. Moshe, F. Onat, S. Cavdar. Brain Res. Bull., **87**, 2012, Nos 2–3, 312–318.
[10] Pinault D. Brain Res. Rev., **46**, 2004, No 1, 1–31.
[11] Crabtree J. W. Eur. J. Neurosci., **12**, 1992, No 4, 1352–1361.
[12] Ohara P. T., A. R. Liebreman. J. Neurocytol., **14**, 1985, No 3, 365–411.
[13] Heizmann C. W., K. Braun. Trends Neurosci., **15**, 1992, No 7, 259–264.
[14] Altman J., S. A. Bayer. J. Comp. Neurol., **275**, 1988, No 3, 406–428.
[15] Mitrofanis J. J. Comp. Neurol., **320**, 1992, No 2, 161–181.
[16] Csillik B., A. Mihaly, B. Krisztin-Peva, R. Fenyo, E. Knyihar-Csillik. Neurosci., **165**, 2006, No 3, 575–578.
[17] Celio M. R. Science, **231**, 1986, No 4741, 995–997.
[18] Bozhilova-Pastirova A., B. Landzhov, B. Izacov, L. Malinova, W. Ovtscharoff. Compt. rend. Acad. bulg. Sci., **57**, 2004, No 3, 67–70.
[19] Majak K., B. Berdel, P. Kowianski, J. Dziewiatkowski, M. Lipowska, J. Morys. Folia Neuropathol., **36**, 1998, No 1, 7–14.
[20] Satoh J., T. Tabira, M. Sano, H. Nakayama, J. Tateishi. Acta Neuropathol., **81**, 1991, No 4, 388–395.

Department of Anatomy, Histology and Embryology
Medical University of Sofia
2, Zdrave Str.
1431 Sofia, Bulgaria
e-mail: lmalinova@mail.bg