DOI 10.1007/s11055-016-0321-y
Neuroscience and Behavioral Physiology, Vol. 46, No. 7, September, 2016

Expression of the Serotonin Transporter in the Ventrolateral Part of the Solitary Tract Nucleus in Rats during the Early Postnatal Period in Normal Conditions and in Serotoninergic System Deficiency during the Prenatal Period of Development

L. I. Khozhai

UDC 611.018.8:612.015.348:599.323.4

Translated from Morfologiya, Vol. 148, No. 5, pp. 38–42, September–October, 2015. Original article submitted June 24, 2015.

An immunocytochemical study method was used to investigate the expression of the serotonin transporter (5-HTT) in the ventrolateral part of the solitary tract nucleus in Wistar rats during the early postnatal period (days 5 and 10) in normal conditions (n = 10) and in conditions of prenatal serotonin deficiency (n = 12). During the early postnatal period, the ventrolateral section of the solitary tract nucleus was found to show transient expression of 5-HTT, most marked in the caudal part of the ventral subnucleus. The numbers of neurons synthesizing 5-HTT in the rostral part of the ventral and lateral subnuclei were small and did not change with age. The caudal part of the ventral subnucleus contained a large number of neurons synthesizing 5-HTT on day 5 of the postnatal period, and this number decreased significantly with age. The caudal part of the lateral subnucleus contained a small number of cells expressing 5-HTT, and this number also decreased with age. 5-HTT expression levels were significantly higher in the caudal areas of both the ventral and lateral subnuclei than in the rostral areas. Prenatal serotonin deficiency decreased the numbers of neurons synthesizing 5-HTT in the nuclei studied here.

Keywords: solitary tract nucleus, ventral subnucleus, lateral subnucleus, serotonin transporter.

The serotonin transporter transmembrane protein (5-HTT) is the main regulator of the extracellular serotonin level, mediating its reuptake from the intercellular space and thus determining its transmission level. 5-HTT has been shown to be expressed mainly by serotoninergic neurons [12, 14], 5-HTT localization sites in the neuropil being varicose swellings of terminal processes where neurotransmitter can be synthesized, stored, and released [5, 15, 16], as well as large and small granules detected using an immunocytochemical reaction for 5-HTT, which may be presynaptic 5-HTT binding sites [1, 13, 14]. During the later periods of prenatal development and the first weeks of the postnatal period, some brain formations in mouse-like rodents (the thalamic nuclei, the trigeminal and cochlear nuclei, the hippocampus, and the cortex) show individual neurons expressing 5-HTT which are not part of the serotoninergic system [6, 8, 12]. It has been suggested that these cases represent "transient," short-lived 5-HTT expression by nonserotoninergic neurons [6, 12]. The solitary tract nucleus has a clearly marked viscerotopic organization and receives numerous afferent fibers from the visceral system; it also has zones projecting to various internal organs [10, 11], including those supporting the vitally important cardiovascular and respiratory reflexes [9, 10]. The ventrolateral part of the solitary tract nucleus is part of the dorsal group of respiratory nuclei, consisting mainly of inspiratory bulbospinal neurons projecting to spinal diaphragmatic and intercostal inspiratory motoneurons mediating inspiration [7, 10]. Serotonin is known to have a direct involvement in controlling respiratory functions [6], though 5-HTT expression as a major factor regulating its transmission in the medulla oblongata, like the relationship between expression and serotonin content, has received virtually no study. The aim

Laboratory for Nervous System Ontogeny, Pavlov Institute of Physiology, Russian Academy of Sciences, St. Petersburg, Russia; e-mail: astarta0505@mail.ru.

of the present work was thus to study the expression of the serotonin transporter in the ventrolateral part of the solitary tract nucleus during the early part of the postnatal period in normal conditions and in serotonin deficiency during the prenatal period of development.

Materials and Methods

Experiments were performed using Wistar rats. Animal keeping and all experimental procedures were performed in compliance with the "Regulations for Studies Using Experimental Animals" (USSR Ministry of Health Decree No. 755 of August 12, 1977). Studies employed inhibition of tryptophan hydroxylase (an enzyme involved in serotonin synthesis) with parachlorophenylalanine (PCPA, Sigma, USA). Female rats ($n = 5$) received i.p. PCPA (400 mg/kg) on day 16 of pregnancy, decreasing the endogenous serotonin content to 50–80% during the period at which fetuses form their own serotoninergic system [1, 3]. Rat pup brains were examined on postnatal days 5 and 10. Controls consisted of animals at the same developmental stages born to intact mothers ($n = 3$). Each time point used 5–6 experimental and 5–6 control rat pups. Specimens were fixed in zinc-ethanol-formaldehyde in phosphate-salt buffer pH 7.4, embedded in paraffin using standard methods, and serial sections of the medulla oblongata of thickness 4–5 μm were cut. The rostral and caudal areas of the ventral and lateral subnuclei of the solitary tract nucleus were examined. The rostral areas of the ventral and lateral subnuclei were studied at the level of the facial nucleus (bregma – 11.64 mm) and the caudal part at the level of the nucleus ambiguus (bregma – 12.00–12.84 mm).

5-HTT was detected using primary anti-serotonin transporter antibody (AbCam, UK) and secondary reagents from an LSAB2 System-HRP kit (Dako, Denmark). Reaction product was visualized using the chromogen DAB+ (Dako, Denmark). After the immunocytochemical reaction, some sections were counterstained with Mayer's hematoxylin (Bio-Optica, Italy) or thionine (Serva, USA, Germany) and embedded in synthetic Permount medium (Termo, USA). The conditions for the imunocytochemical reaction were standardized and all immunostaining procedures were applied to histological sections of the medulla oblongata from intact and experimental animals at different time points simultaneously. Analysis of the reaction results considered staining of the neuron cytoplasm, the presence of terminal varicose swellings, and the distribution of immunopositive granules – as suggested 5-HTT binding sites [12].

Morphological analysis and counting of neurons were performed using digital images of serial sections of different parts of the subnuclei studied, obtained using a Leica DME (Leica, Germany) light microscope and a Leica EC3 digital camera. Numbers of immunopositive neurons were counted in standard areas of 0.1 mm^2. Data were analyzed statistically in Statistica 6.0, ImageScope Color, and Origin 50. Significant differences were identified using Student's t test. Differences were regarded as significant at $p < 0.05$.

Results

Rostral parts of the ventral and lateral subnuclei of the solitary tract nucleus. On days 5 and 10, the rostral part of the ventral subnucleus in control animals showed 2.4 ± 0.8 and 2.0 ± 0.7 immunopositive neurons per standard section area respectively. On day 5, quite high densities of plexuses of processes and small varicose swellings were visualized in the neuropil (Fig. 1, *a*, *b*). By day 10, there was a decrease in the staining intensity of the neuron cytoplasm, with a reduction in the number of terminal varicose swellings, along with the appearance of immunopositive dust grains.

On days 5 and 10 with prenatal serotoninergic system deficiency, the dynamics of changes in the rostral part of the ventral subunit corresponded to those in controls. Immunopositive neuron counts in the subnucleus were 2.0 ± 0.4 and 1.1 ± 0.9, respectively. By day 10, as in controls, there was some decrease in the intensity of the immune reaction in the neuron cytoplasm, with a drop in the number of terminal varicose swellings and the appearance of immunopositive dust grains.

On days 5 and 10, the rostral part of the lateral subnucleus in control animals showed insignificant numbers of immunopositive neurons – 1.3 ± 0.5 and 1.0 ± 0.7, respectively. On day 5, the density of plexuses of processes in the neuropil and the positions of terminal varicose swellings were greater than on day 10. In prenatal serotoninergic system deficiency, the numbers of immunopositive cells in the lateral subnucleus on days 5 and 10 were 0.8 ± 0.3 an 0.60 ± 0.10, respectively. The dynamics of changes in the neuropil corresponded to that in controls.

Thus, in control animals, there were very few 5-HTT-expressing neurons the rostral part of the ventral and lateral subnuclei of the solitary tract nucleus and their numbers did not change over the study period. In experimental rats, as in controls, the intensity of 5-HTT expression was minor and the numbers of immunopositive neurons corresponded to those in controls.

The caudal parts of the ventral and lateral subnuclei of the of the solitary tract nucleus. On day 5, the caudal part of the ventral subunit in control animals contained 8.3 ± 1.4 immunopositive neurons. The neuropil showed a dense plexus of immunopositive fine terminal processes with multiple varicose swellings and grains on their surfaces (see Fig. 1, *c*, *d*). By day 10 (see Fig. *1*, *e*, *f*), the number of immunopositive neurons decreased significantly to 5.1 ± 1.5, though the density of the network of processes in the neuropil was essentially the same as that on day 5.

On day 5, the caudal part of the ventral subnucleus in experimental animals contained 4.7 ± 1.4 immunopositive neurons (in the central sections). The neuropil showed a quite dense plexus of processes with multiple varicose swellings and granules. On day 10, the number of immunopositive neurons corresponded to that on day 5, at 4.5 ± 0.8. The morphological pattern in the neuropil was similar to that on day 5.

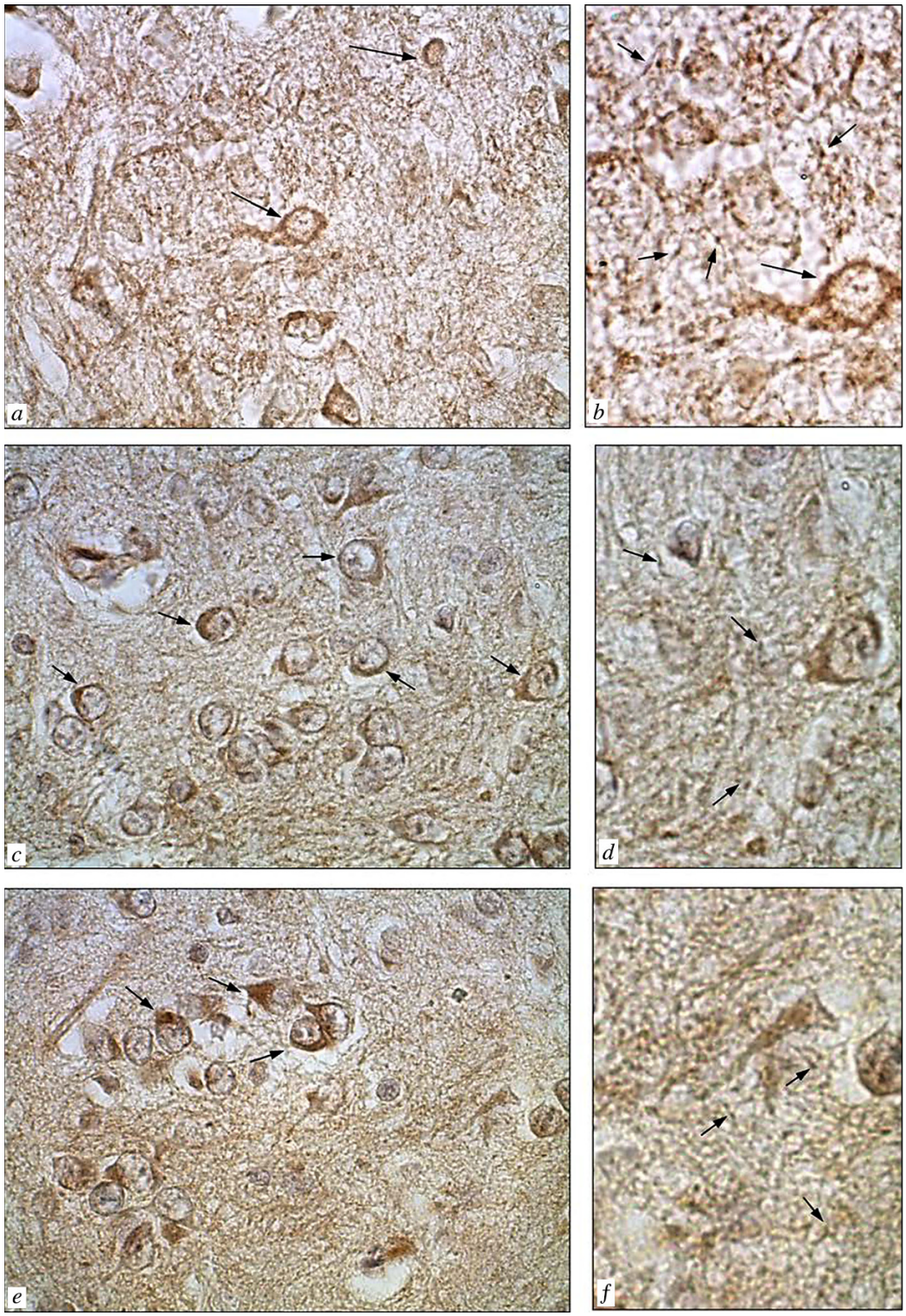

Fig. 1. Rostral (*a*, *b*) and caudal (*c*–*f*) parts of the ventral subunit of the solitary tract nucleus in rat pups on days 5 (*a*–*d*) and 10 (*e*, *f*) after birth. *a*, *b*) Immunopositive neurons (long arrows); *b*) enlargement of fragment of Fig. 1, *a*; terminal varicose swellings (short arrows); *c*) immunopositive neurons (arrows); *d*) enlargement of fragment of Fig. 1, *c*. Neuropil, terminal varicose swellings (arrows); *e*) immunopositive neurons (arrows); *f*) enlargement of fragment of Fig. 1, *e* – terminal varicose swellings (arrows). Immunohistochemical reaction for 5-HTT. Objective ×100, ocular ×10.

On day 5, the caudal part of the lateral subnucleus in control animals contained 3.1 ± 1.1 immunopositive neurons per standard section area. The neuropil contained a dense plexus of terminal processes and multiple varicose swellings and granules. By day 10, the number of immunopositive neurons corresponded to that on day 5, at 2.8 ± 0.9, though the density of the plexus of processes and varicose swellings in the neuropil decreased.

On days 5 and 10, the caudal part of the lateral subnucleus in experimental rats showed significant decreases in the number of immunopositive neurons, to 1.3 ± 0.6 and 1.0 ± 0.7, respectively. The density of the plexus of processes and varicose swellings in the neuropil also decreased from that in controls.

Discussion

The experiments reported here showed that during the early postnatal period, the dynamics of 5-HTT expression in the rostral and caudal parts of the ventrolateral segment of the solitary tract nucleus were different.

In control and experimental animals, the numbers of neurons synthesizing 5-HTT in the rostral parts of the ventral and lateral subnuclei of the solitary tract nucleus were low and did not change with age. The level of 5-HTT expression was probably low and prenatal serotonin deficit had no marked effect.

In the caudal part of the ventral subnucleus, control animals on day 5 after birth had quite large numbers of 5-HTT-synthesizing neurons, which is evidence for intense 5-HTT expression, though the number of these neurons decreased sharply with age (day 10, almost twofold). The present results showed that during the early postnatal period, the number of 5-HTT-expressing neurons in this area of the subnucleus in conditions of serotonin deficiency decreased about two-fold and remained the same to day 10. These changes are probably secondary in nature and result either from impairments to synthetic processes resulting from delayed neuron differentiation or reductions in afferent and efferent connections as a result of underdevelopment of their targets.

In control animals, the number of 5-HTT-expressing neurons in the caudal part of the lateral subnucleus on day 5 after birth was small, and underwent a slight but significant decrease by 10 days; experimental rats showed occasional neurons of this type with no change in number with age.

In the caudal parts of both the ventral and lateral subnuclei, the numbers of 5-HTT-expressing neurons were 3–3.5 times greater and the level of 5-HTT expression here was significantly greater than in the rostral parts.

These observations would appear to reflect the different functional activities of neurons in the rostral and caudal parts of the subnuclei, though note should be taken of the structural and organizational characteristics of interneuronal connections in this part of the medulla oblongata.

The ventrolateral part of the solitary tract nucleus has been shown to have a variety of incoming visceral afferent projections and descending projections from above-lying parts of the brain. Important information for controlling respiration arrives at the caudal parts of the respiratory subnuclei of the solitary tract nucleus both via afferent fibers from various structures involved in the process of respiration and the vagus nerve [11]. Furthermore, widespread efferent connections from neurons in the caudal part of the ventrolateral area have been established with the nucleus ambiguus and nucleus retroambiguus (innervating the musculature of the bronchi, heart, and lungs involved in controlling the phases of the respiratory cycle), which are among the ventral respiratory group of nuclei, and with the nuclei of the spinal cord [2, 9].

The rostral part, conversely, showed a small number of neurons sending projections to the intercostal respiratory muscles [4].

Thus, the results obtained here show that during the early period of postnatal development, the ventrolateral part of the solitary tract nucleus showed transient expression of the serotonin transporter, which was most marked in the caudal part of the ventral subunit. The presence of the transporter suggests serotonin transmission which, considering the multitude of afferent and efferent connections with neurons in the nuclei of the ventral respiratory group and the spinal cord nuclei, may take part in regulating the mechanisms of the phases of the respiratory cycle. Impairments to the expression of the serotonin transporter in the ventral and lateral subnuclei during the early postnatal period can probably elicit decreases in the level of serotonin transmission controlling the activity of the inspiratory neurons of this subnucleus, which may in turn be the cause of respiratory impairments in neonates, leading to apnea and sudden infant death syndrome.

This study was supported by the Russian Foundation for Basic Research (Grant No. 15-04-02167).

REFERENCES

1. E. V. Naumenko, *Central Regulation of the Piuitary-Adrenal Complex*, Nauka. Leningrad (1973).
2. S. L. Olenov and A. S. Olenev, *Neurobiology-95*, St. Petersburg State Pediatric Medical Academy, St. Petersburg (1995).
3. N. K. Popova, E. M. Nikulina, V. N. Arav, and N. N. Kudryavtseva, "The role of serotonin in one type of aggressive behavior," **61**, No. 2, 183–186 (1975).
4. M. V. Sergievskii, N. Ya. Kireeva, and N. A. Gordievskii, "Possible interactions between the inspiratory and expiratory neuron systems," *Ros. Fiziol. Zh.*, **69**, No. 4, 439–444 (1983).
5. L. F. Agnati, G. Guidolin, M. Guescini, et al., "Understanding wiring and volume transmission," *Brain Res. Rev.*, **64**, No. 1, 137–159 (2010).
6. G. L. Brüning and O. Liangos, "Transient expression of the serotonin transporter in the developing mouse thalamocortical system," *Acta Histochem.*, **99**, No. 1, 117–121 (1997).
7. L. Fedorco, E. G. Merrill, and J. Lipski, "Two descending medullary inspiratory pathways in phrenic motoneurones," *Neurosci. Lett.*, **43**, 74–77 (1984).
8. S. R. Hanson, E. Mezey, and B. J. Hoffman, "Serotonin transporter messenger RNA in the developing rat brain: early expression in se-

rotonergic neurons and transient expression in nonserotonergic neurons," *Neuroscience*, **83**, No. 4, 1185–1201 (1998).
9. E. Merrill, "The lateral respiratory neurons of the medulla: their association with nucleus ambiguous, nucleus retroambigualis, the spinal accessory nucleus and the spinal cord," *Brain Res*., **24**, No. 1, 11–28 (1970).
10. J. F. Paton, "Pattern of cardiorespiratory afferent convergence to solitary tract neurons driven by pulmonary vagal C fiber stimulation in the mouse," *Am. J. Neurophysiol*., **79**, No. 5, 2365–2373 (1998).
11. C. G. Wilson, Z. Zhang, and A. C. Bonham, "Non NMDA receptors transmit cardiopulmonary C fiber input in nucleus tractus solitarii in rats," *J. Physiol*., **496**, No. 3, 773–785 (1996).
12. F. C. Zhou, Y. Sari, and J. K. Zhang, "Expression of serotonin transporter protein in developing rat brain," *Dev. Brain Res*., **119**, 33–45 (2000).
13. F. C. Zhou, J. H. Tao-Cheng, L. Segu, et al., "Serotonin transporters are located on the axons beyond the synaptic junction: anatomical and functional evidence," *Brain Res*., **805**, 241–245 (1998).
14. F. C. Zhou, Y. Xu, S. Bledsoe, et al., "Serotonin transporter antibodies: production, characterization, and localization in the brain," *Mol. Brain Res*., **43**, 267–278 (1996).
15. M. L. Zoli and L. F. Agnati, "Wiring and volume transmission in the central nervous system: the concept of closed and open synapses," *Prog. Neurobiol*., **49**, No. 4, 363–380 (1996).
16. M. L. Zoli, C. Torri, R. Ferrari, et al., "The emergence of the volume transmission concept," *Brain Res. Brain Res. Rev*., **26**, No. 2–3, 136–147 (1998).