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Original Articles

The Bed Nucleus of the Stria Terminalis: Cytoarchitecture and Morphometry of its Subnuclear Organizationin the rat Brain

Albert Gradev[1], Lazar Jelev[1], Nikolai Lazarov[1,2]*

[1]Department of Anatomy, Histology and Embryology, Medical University of Sofia, Sofia, Bulgaria
[2]Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria

*Corresponding author e-mail: a.gradev@medfac.mu-sofia.bg

The complexity of the bed nucleus of the stria terminalis (BNST), its function, sex difference, cyto- and chemoarchitectonic structure has intrigued scientists during the last decades. As part of the extended amygdala, it is involved in many limbic functions being the main target of treating anxiety and addiction as lately revealed. This paper is a brief review of the BNST structure and also a morphometric study of its subnuclear groups in the adult male rat brain. The Kluver-Barrera staining revealed the main white matter tracts which serve us for landmarks to identify the BNST subdivisions. The majority of BNST neurons were small in size, less than 15μm in diameter, oval in shape and with prominent nucleoli. The present results clearly show the complexity in the BNST structural organization to better understand its function, and to emphasize it as a novel research area of the functional morphology.

Key words: Bed nucleus of stria terminalis, morphometry, cytoarchitecture, rat

Introduction

The bed nucleus of the str ia terminalis (BNST) is a complex brain structure located in the basal forebrain which belongs to the extended amygdala [1, 23]. Its position is almost the same in the rodent and human brain [35, 43] and can be defined as a structure that surrounds the anterior commissure, medial to the internal capsule, posterior to the

nucleus accumbens, anterior to the thalamus and in proximity to the ventral pallidum and hypothalamus [2]. Such a location gives a bright suggestion for its function as a general integral unit for the limbic system and makes it easily accessible for different studies. Based on its complexity, a number of classification systems about the subnuclear groups of the BNST has been developed [14, 26, 32, 35, 37]. It is generally accepted that the BNST is primarily divided into anterior and posterior divisions. The anterior division consists of dorsal and ventral areas and contains a number of nuclei, including the oval, fusiform, rhomboid, magnocellular, ventral and dorsomedial nucleus. The principal, transverse, supracapsular (strial extension) and interfascicular nuclei can be identified in the posterior part [37].

In this study, we further describe the nuclear organization of the BNST in the adult rat brain.

Material and Methods

Ten 3-month-old male Wistar rats were used for the study. The experiments were performed in agreement with the European Communities Council Directive 2010/63/EU for the protection of animals used for scientific purposes. After deep ether anesthesia, the animals were euthanized by cervical dislocation and then transcardially perfused. After washing the blood vessels with cold normal saline (0.9% NaCl solution) for about 20 min, 4% paraformaldehyde fixation solution was introduced for about 30 min. After decapitation, the brains were quickly removed from the skull and postfixed overnight with 4% paraformaldehyde in a refrigerator at 4°C. The brain was then trimmed into blocks at the levels of the BNST as follows: coronal section of the whole brain at a level 5 mm posterior to the olfactory tubercle; coronal section of the hemisphere (thickness of 4 mm) – from 1 mm anteriorly to 3 mm posteriorly to the anterior commissure; sagittal section of the hemisphere (thickness of 3 mm) – 0.5 mm laterally to the midline; horizontal sections of the hemisphere (thickness of 4 mm) – from 1 mm under to 3 mm above the anterior commissure.

Paraffin embedded tissue slices from specific brain regions were cut at a thickness of 7 μm and appropriately stained to demonstrate the brain structures. We used routine H&E and Nissl stains to visualize the neuronal morphology while Kluver-Barrera staining method [29] was applied to observe both myelinated fibers and nerve cells. The samples were examined and photographed under an Olympus light microscope, digital images were created with a slide scanner (Olympus VS 120 Slide scanner) and an Imaging Analyzing System (Olympus VS-ASW) was used for morphometry. The BNST subdivisions are mapped according to Paxinos and Watson's rat brain in stereotaxic coordinates [35] and the rat brain schematics available from Swanson [37].

Results

On the frontal, sagittal and horizontal slices, we found a large number of neurons in the different regions in the BNST (**Figs. 1-3**). We also observed very clearly the transition between the anterior and posterior nuclear groups in horizontal and sagittal sections through the stria terminalis (**Fig. 1**). In the anterior division, the

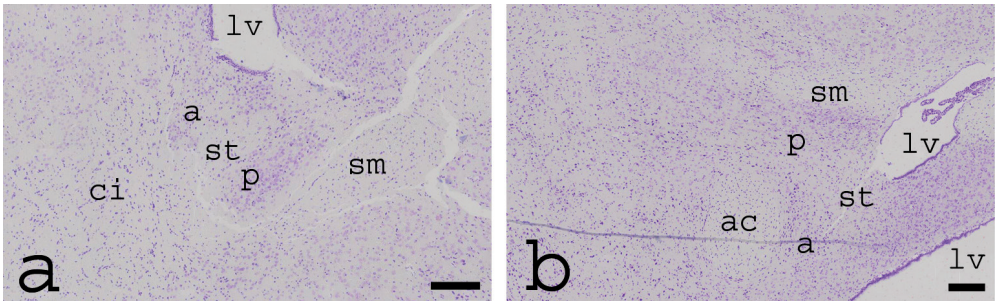

Fig. 1. Photomicrographs of Nissl-stained horizontal (a) and sagittal (b) sections at the level the BNST. The stria terminalis (st) divides the nucleus into an anterior (a) and a posterior (p) parts. Stria medullaris (sm), capsula interna (ci), and lateral ventricle (lv) are also visible while the anterior commissure is seen only on (b). Scale bar = 200 μm.

posterodorsal nucleus of the BNST, also called oval nucleus, is shown on **Fig. 2**. It is a part of anterolateral area according to the atlas of Swanson [37]. The oval nucleus was composed of small-sized neurons with an oval to polygonal shape and diameter of 9-15 μm that cover an area of approximately 120 μm^2. The neurons have large nuclei (7-13 μm in diameter) with a prominent nucleolus. The Nissl bodies were well distinguished, which correlates with the great metabolic activity of these neurons. In the anterior part several nuclei were identified – the ventromedial, ventrolateral nucleus, medialis anterior, lateralis posterior, or the anteromedial area and anterolateral area according to Swanson [37]. In the anterolateral area with its ventral part, we observed neurons of various shapes and size and most of these showed oval bodies (**Fig. 2**). We also found a small number of spindle-shaped perikaryal profiles. The morphometric analysis revealed a population of small-sized cells with diameter ranges from 8 to 16 μm that occupied a cell surface area of approximately 100 μm^2. The boundaries between the other different areas or nuclei were not clearly

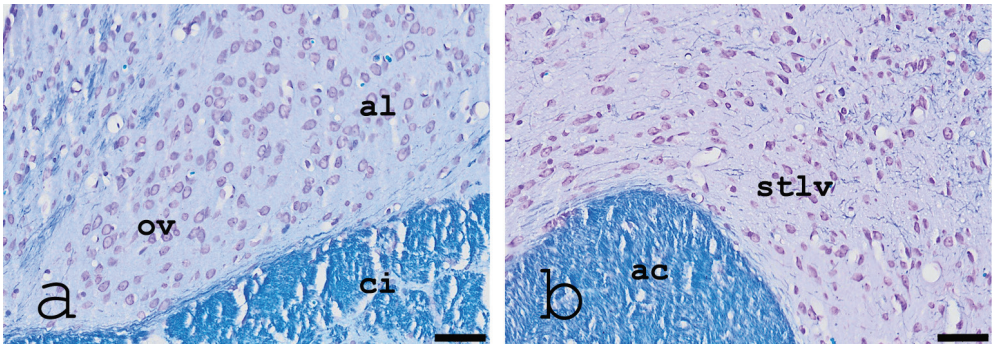

Fig. 2. Photomicrographs of Kluver-Barrera-stained frontal sections through the BNST. In (a) the oval nucleus (ov) is presented. Note the blue-depicted initial fibers of stria terminalis which are seen between the neurons. The capsula interna (ci) and the remaining part of anterolateral area (al) are evident as well. In (b) the neurons in the ventrolateral area (stlv) are shown. The anterior commissure (ac) is also visible. Scale bar = 50 μm.

discernible. In the posterior division of BNST, we identifed the BNSTprincipal and supracapsular nuclei. In the supracapsular nucleus, also known as the strial extension of the BNST, a small number of small-to-medium in size, oval to polygonal neurons with a diameter between 8 μm and 15 μm, and a cell surface area of 115-191 μm^2 was seen. The neurons also had large nuclei (6-12 μm in diameter) with prominent nucleoli. The nerve fibers of the stria terminalis, depicted in blue after the Kluver-Barrera staining, were clearly visible between neurons. In the superior portions of the principal nucleus of the BNST and initial parts of the strial extension, i.e. the supracapsular nucleus on transverse and sagittal sections, we found a few small-sized, lightly-stained neurons with a diameter 8-13 μm and a surface area ranging from 50 μm^2 to 89 μm^2 (**Fig. 3**).

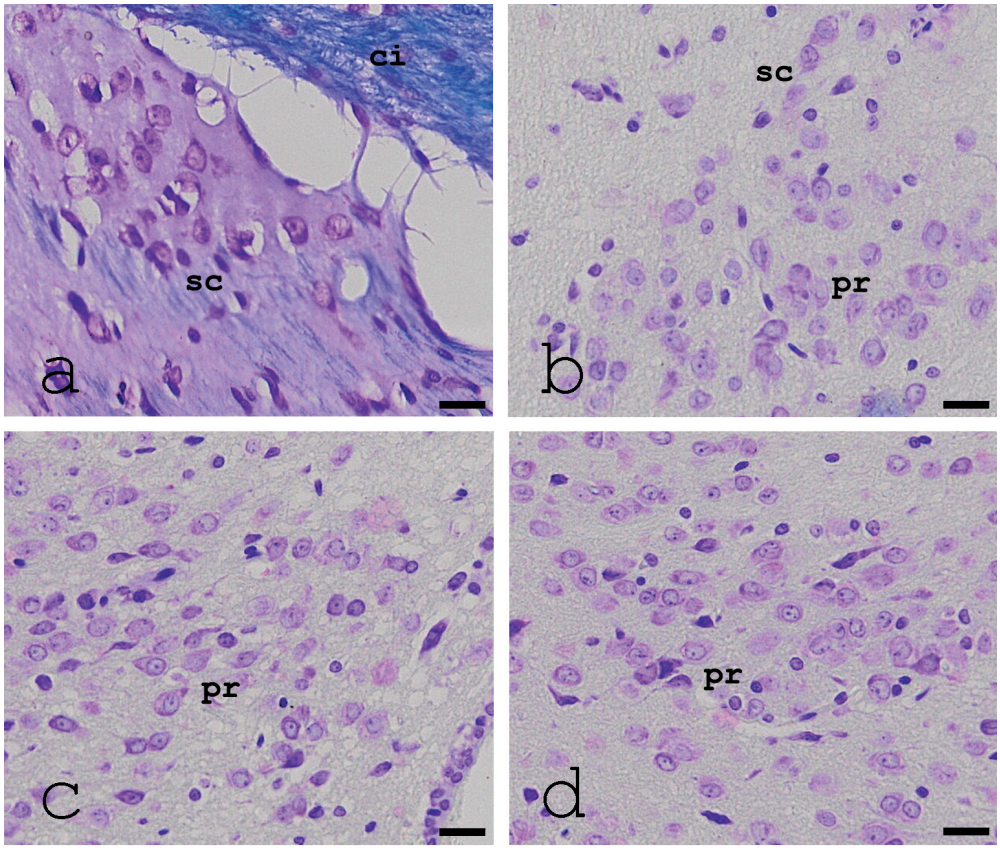

Fig. 3. Photomicrographs of a Kluver-Barrera-stained coronal section (a), and Nissl-stained horizontal (b) and sagittal (c,d) sections of the BNST. The supracapsular nucleus (sc) is shown in (a). The blue-stained fibers of the stria terminalis and the capsula interna (ci) are displayed. In (b) neurons in the principal nucleus (pr) and initial portion of the supracapsular nucleus (sc) are identified. In (c) and (d) the neurons of the principal nucleus (pr) at lower levels are shown. Scale bar = 20 μm.

Discussion

The BNST is originally described by Johnston [25] as a cell cluster surrounding the initial part of the stria terminalis. Subsequently, it has been extensively studied but the interest for it as a possible contributor to some psychiatric disorders has been renewed in the last 20 years [4, 11, 12, 16, 17, 18, 21, 22, 31, 44]. Evidence for its complexity is obvious in the works of different morphologists, who have described a number of subnuclei and specific neuronal types [26, 35, 37]. The divisions of the BNST are based on morphological (location to anterior commissure or stria terminalis), functional or neurochemical criteria [14, 26, 32, 35, 37].

Complexity of the BNST is clearly shown in many studies, most of which on the rat brain, although they appear contradictory in relation to its subnuclear structure [7, 14, 26, 32, 35, 37]. Classically, the BNST is divided into medial and lateral subdivisions [1, 32], which corresponds to the theory of the extended amygdala [7, 23]. Another major classification divides the BNST in antero-posterior direction [26, 37]. This division is consistent with its embryonic origin and is more useful with regards to its connections [5, 20]. Another way to divide the nuclear groups within the BNST is the dorso-ventral orientation with respect to the anterior commissure [14]. Additionaly each division displays its own subnuclei. Over the time, many neuroscientists have used the atlases of Paxinos and Watson [35] or Swanson [37] and the terminology therein. In the latter, an improved version of the Ju and Swanson classification was added [26]. In the further descriptions the Swanson nomenclature [37] and Bota's table [7] for comparison of terms are used.

Of all nuclear groups, the principal nucleus, the oval nucleus, and some of the medioventral and ventrolateral nuclei are particularly fascinating due to the fact that some of them exhibit different characteristics between sexes and thus show sexual dimorphism [3, 15, 27, 39, 40]. The sex difference starts after birth, and continues into adulthood [8]. It is a result of apoptosis [9], which might be epigenetically controlled [33]. Some of the neurons might continue their development and adapt their cellular and synaptic activity during puberty, depending on previous stress history [18, 22, 42]. This fact, together with the current evidence for stem cell niches in the ventral pallidum [22], indicates that the BNST is very dynamic in its development. This dynamic, taking into account the function of the nucleus, bring us to the idea of future in-depth studying of periadolescent development, and specifically how stress factors could correlate with different psychiatric disorders, and how do they change the anatomy of the BNST.

The complexity of BNST is not only valid for its subnuclear division, but also for its neurochemical profile, types of neurons and their connections, functions and gene expression [5, 6, 12, 14, 26, 32, 34]. The main types of neurons in BNST are GABAergic, mainly seen in the oval nucleus [28, 36]. A few BNST neurons in its principal, medioventral and fusiform nuclei use glutamate as a neurotransmitter [28, 36]. A broad spectrum of neuropeptides including corticotropin-releasing hormone, neuropeptide Y, enkephalins, dynorphin, neurotensin, oxytocin, pituitary adenylate-cyclase-activating polypeptide, vasoactive intestinal peptide, cholecystokinin, substance P, orexin and galanin has also been established in the nucleus [6, 23 30, 31]. Among them, oxytocin and arginine vasopressin are the most intriguing since they are proposed as novel medication treatments for some anxiety disorders [13]. This finding suggests a brain-storming complexity of the limbic connectivity, circuits and controls of emotions. In

addition, in the BNST of some monogamous rodent species, such as the California mouse, some oxytocinergic neurons were recently identified [17] and they belong to the so-called extra-hypothalamic oxytocin system. Moreover, many oxytocin (OXT) receptors are present on BNST-neurons [11, 15, 19, 38, 41]. The occurrence of such OXT receptors on GABAergic terminals, some of them co-expressed with corticotropin-releasing hormones, a neuropeptide [6, 10, 11], suggests a large contribution of OXT to all functions of the BNST, and primarily the anxiety-related ones. Taken together with its sexual dimorphism and intriguing peripubertal development, the present data identify the BNST as a novel research area, particularly in the field of functional morphology and psychiatry.

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