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The Astrocytic Environment Differs among the Divisions of the Rat Striatum

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The current concept of the structure of the striatum is that it consists of a dorsolateral, sensorimotor, and a ventromedial, limbic part. This division is backed up by studies of the neuronal population of the striatum. In this investigation we aim to elucidate the morphological basis of the different striatal areas by studying their glial environment. For this purpose, we employed immunohistochemistry for GFAP for visualization of astrocytes, followed by image analysis for quantitative assessment of astroglia in different areas of the striatum. Our results show that the astroglial parameters in the dorsolateral division of the striatum are considerably greater than in the ventromedial one. This peculiarity of the glial environment hints towards the notion that astroglia is differentially regulated according to local characteristics of synaptic activity and density in different divisions of the striatum.

Key words: striatum, astrocytes, astroglia, GFAP

Introduction

The striatum of the rat is a relatively large structure, which occupies much of the volume of the telencephalic hemispheres [9]. It has been subdivided into a dorsal (or dorsolateral) and ventral (or ventromedial) portion. The dorsal part is usually accepted to include the caudate-putamen complex, while the ventral part is associated with the nucleus accumbens and the olfactory tubercle [12]. However, no sharp boundary between the two divisions exists, neither histologically nor immunohistochemically [10].

The main cellular population of the striatum are the medium spiny neurons, which, through their intricate dendritic tree, are the target of extrinsic afferents [2]. The source of these afferents might be regarded as the most reliable criterion for dividing the striatum. Projections from the cortex, amygdala and thalamus reach the neurons in different parts of the striatum [4, 5, 11]. Thus, the dorsolateral part of striatum is integrated in the sensorimotor circuitry, the ventromedial is visceral-related, and areas lying between these extremes receive associational information [10].

Despite the knowledge about the neuronal connections of the divisions of the striatum, little has been described about their glial environment. It is well known that as-

troglial cells are intimately associated with synapses. They are stimulated by increased synaptic activity, and can regulate synaptic transmission themselves [3, 8]. Therefore, information about the astroglia of the striatum can shed light on some functional aspects of this brain structure, and the regulation of the activity in its divisions.

Aim

In the present study we aim to investigate the astrocytic population of the rat striatum, comparing the dorsal and the ventral part, by means of a quantitative assessment of an immunohistochemical staining.

Materials and Methods

We used 9 adult male Sprague-Dawley rats, kept in cages under standard conditions, with free access to food and water. After terminal anaesthesia with ketamine and xylazine, the animals were fixed to a styrofoam board and perfused transcardially with 300 ml ice-cold 4% solution of paraformaldehyde in phosphate-buffered saline. Following perfusion, brains were removed and postfixed in the same fixative overnight. Upon cryoprotection in 20% sucrose the brains were sectioned in 40 μm sections on a freezing microtome and processed for immunohistochemistry.

The resulting free-floating sectioned were incubated for 30 min in a solution containing 3% H_2O_2 for quenching the endogenous peroxidase activity. Blocking of the non-specific immunoreactivity was obtained with incubation in 3% bovine serum albumin with addition of 0,3% Triton X-100. Antibody against GFAP (rabbit; Abcam, ab7779) was used as a primary antibody in a 1:800 dilution. The sections were incubated with the primary antibody for 12 h. After washing, the sections were incubated with the secondary antibody (anti-rabbit biotinilated IgG; Dako, E0432) for 2 h. Vectastain ABC HRP Kit was used per manufacturer's directions and 3,3-diaminobenzidyne was employed as a chromogen for visualization of the reaction. The free-floating sections were mounted on glass slides, air-dried, dehydrated and coverslipped. In order to evaluate the specificity of the immunohistochemical reaction, sections were processed in control experiments in which the primary antibody was omitted, the secondary antibody was omitted, the ABC-reagent was omitted, or non-immune antiserum was used in place of the anti-GFAP antiserum.

Digital images were obtained using a Leica DM1000 microscope coupled with a standard camera. Care was taken that light conditions and exposure time did not vary between frames. The obtained images were analyzed using the software package ImageJ (NIH, USA). For the analysis only zones, clearly belonging to either the dorsal or the ventral division of the striatum were used; zones from intermediate areas were avoided. Cell density was determined by manually counting cell bodies in a given zone. Immunopositive area was calculated as a percentage of pixels of all pixels in the same zone. Pixels were considered immunopositive when they had gray values above a threshold obtained according to the algorithm of Otsu [7]. Statistical analysis was performed using GraphPad Prism 9. Student's t-test was applied, $p<0.05$ was considered statistically significant.

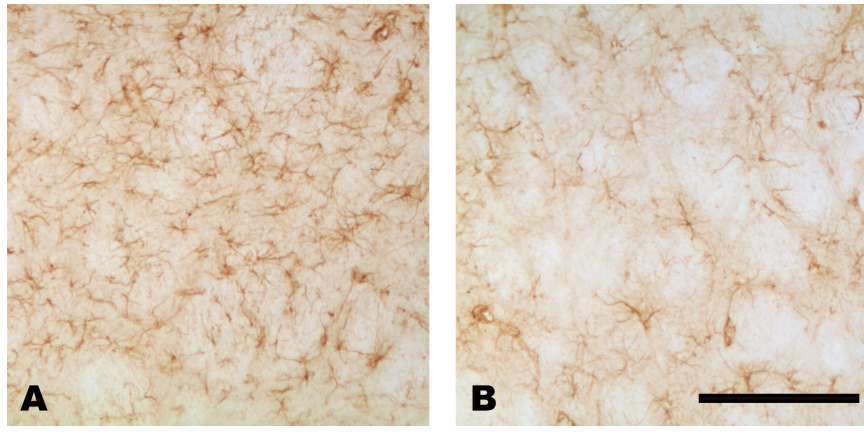

Fig. 1. Representative images of GFAP-stained astrocytes of the dorsal (A) and ventral (B) striatum. Scale bar = 100 μm

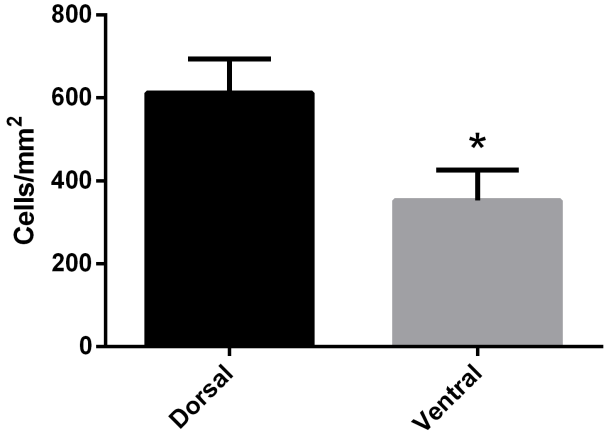

Fig. 2. Comparison between the density of GFAP+ cells per mm^2 area in the dorsal and ventral striatum. Data represents average values ±SD. Student's t-test. Asterisk indicates $p < 0.05$

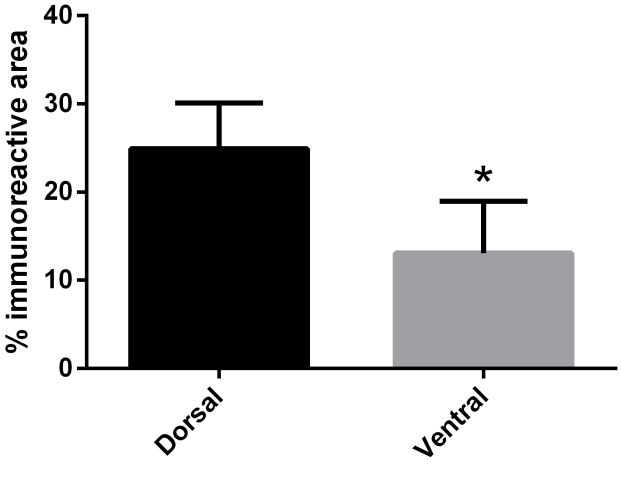

Fig. 3. Comparison between GFAP-immunopositive area in the dorsal and ventral striatum. Data represents average values ±SD. Student's t-test. Asterisk indicates $p < 0.05$

Results

Astrocytes were visualized throughout the striatum. They were seen extending their processes in all directions, covering a territorial domain in the gray matter. In some areas, processes of neighboring cells were seen overlapping and crossing, but no cellular clustering could be demonstrated. Some astrocytes wrapped their bodies and processes around the fibers of the internal capsule. GFAP+ elements were seen wrapping around blood vessels of different caliber, running in different directions in all parts of the striatum.

A clear-cut boundry between any of the divisions of the striatum could not be established. However, a gradient of the amount of stained elements was evident. The zones in the most dorsal part of the striatum, immediately below corpus callosum, showed a dense cell population (Fig. 1A). Contrary, astrocytes in the most ventral parts were scarce and divided by large zones of immunonegative tissue (Fig. 1B).

The quantitative assessment confirmed this finding. The dorsal division of the striatum contained significantly more cells (Fig. 2), and the immunoreactive area was larger, compared to the ventral division (Fig 3).

Discussion

Our findings generally confirm the notion, that the striatum is a structure relatively scarcely populated by astrocytes [6]. When stained for GFAP, the striatum looks relatively pale compared to the neighboring structures. This can be partially explained by the fact, that despite the numerous neuronal population, the synaptic density is not as great, or at least nor all synapses are active at the same time [6]

The astrocytes are not uniformly distributed in all parts of the striatum. Despite this, no unambiguous delineation between dorsal and ventral striatum can be made solely by their distribution. It is clear however, that different divisions of the striatum posses different astrocytic populations. This can be interpreted in the context of neuron-glia interaction. Astrocyte-rich environment could correlate with greater synaptic activity [3]. Therefore, this study presents an indirect evidence for differences in synaptic density and/or activity in different parts of the striatum.

Moreover, the possible differences of the capacity for glial scarring should be taken into account. It is widely known that injection of substances/cells in the striatum may produce different results, depending on the exact coordinates used [1]. The extent of astrocytic activation in such experimental models should be considered.

Conclusion

The rat striatum is a heterogeneous structure, despite the very subtle histological differences. Astrocytes are not uniformly distributed in the tissue of the different divisions of the striatum. This could have functional implications, considering neuron-glia interactions.

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