ORIGINAL ARTICLE

UDC: 611.085::[611.81:611.018.83
DOI: 10.2298/VSP1108655S

Neurons of human nucleus accumbens

Neuroni humanog nukleusa akumbensa

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Abstract

Background/Aim. Nucleus accumbens is a part of the ventral striatum also known as a drug active brain region, especially related with drug addiction. The aim of the study was to investigate the Golgi morphology of the nucleus accumbens neurons. **Methods**. The study was performed on the frontal and sagittal sections of 15 human brains by the Golgi Kopsch method. We classified neurons in the human nucleus accumbens according to their morphology and size into four types: type I – fusiform neurons; type II – fusiform neurons with lateral dendrite, arising from a part of the cell body; type III – pyramidal-like neuron; type IV – multipolar neuron. The medium spiny neurons, which are mostly noted regarding to the drug addictive conditions of the brain, correspond to the type IV – multipolar neurons. **Results.** Two regions of human nucleus accumbens could be clearly recognized on Nissl and Golgi preparations each containing different predominant neuronal types. Central part of nucleus accumbens, core region, has a low density of impregnated neurons with predominant type III, pyramidal-like neurons, with spines on secondary branches and rare type IV, multipolar neurons. Contrary to the core, peripheral region, shell of nucleus, has a high density of impregnated neurons predominantly contained of type I and type IV – multipolar neurons, which all are rich in spines on secondary and tertiary dendritic branches. **Conclusion.** Our results indicate great morphological variability of human nucleus accumbens neurons. This requires further investigations and clarifying clinical significance of this important brain region.

Key words:
nucleus accumbens; neurons; dendrites; anatomy; histology.

Apstrakt

Uvod/Cilj. Nukleus akumbens je deo ventralnog strijatuma, poznatog kao region mozga koji je osetljiv na dejstvo opijata, pogotovo na bolesti zavisnosti. Cilj ove studije bio je istraživanje Goldži morfologije neurona humanog nukleusa akumbensa. **Metoda.** Studija je izvedena na frontalnim i sagitalnim presecima na 15 humanih mozgova prema Goldži Kopš metodi. Klasifikovali smo neurone humanog nukleusa akumbensa prema morfologiji i veličini na četiri tipa: tip I – fuziformni neuron; tip II – fuziformni neuron sa bočnim dendritom koji izlaze sa bočne strane tela neurona; tip III – telo neurona slično piramidi; tip IV – multipolarni neuron. **Rezultati.** Neuroni srednje gustine spina, koji su najviše vezani za osobine adikcije ovog regiona, bili su vezani za tip IV – multipolarni neuron. Dva regiona nukleusa akumbensa mogla su biti jasno razgraničena na Nisl i Goldži preparatima, a svaki je sadržavao dominantne neuralne tipove. Centralni deo nukleusa akumbensa, srž nukleusa akumbensa, imao je malu gustinu impregnisanih neurona sa predominantnim tipom III, piramidi sličnim telom neurona sa spinama na sekundarnim granama i retkim tip IV, multipolarnim neuronima. Suprotno od srži, periferni region, kora nukleusa akumbensa, imao je visoku gustinu impregnisanih neurona dominantnih po tipu I, fuziformnih, i tipu IV, multipolarnih neurona, sa velikom gustinom spina na sekundarnim i tercijarnim dendritima. **Zaključak.** Naši rezultati pokazali su veliku morfološku varijabilnost humanog nukleusa akumbensa. Ovo zahteva dalja istraživanja i pojašnjavanje kliničkih značajnosti ovog važnog moždanog regiona.

Ključne reči:
nukleus akumbens; neuroni; dendriti; anatomija; histologija.

Introduction

Nucleus accumbens occupies ventral part of the striatum, laying laterally on the septum. This nucleus is also known as nucleus accumbens septi. Nucleus accumbens is known to play an important role in pleasure, reward, and addiction.

Subdivisions of nucleus accumbens were described, consisting of the core and shell region. The core region is involved in motor functions and the shell region is involved in

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emotional and motivational processes [1]. Two neurochemical subdivisions described in the human nucleus accumbens, could be related to the core and shell regions [2].

In the beginning of the last century, Ramón and Cajal [3] investigated the morphological changes of the brain, due to neuron structure alterations. Behavioral changes initiated by drugs, altered morphological and biochemical structures of brain plasticity and synaptic connectivity [4, 5]. Recent tracing and histochemical studies provide enough evidence to consider that the medium spiny neurons of the nucleus accumbens have the specific role of striatum relation with its connectivity pattern [6]. Nucleus accumbens has the key role in reward and enforcement neuronal processes *via* glutaminergic afferent pathways originated from the basolateral amygdala, ventral subiculum and medial prefrontal cortex [7, 8]. Stimulated dopamine transmission in human nucleus accumbens is related to the addictive properties and positive reinforcement by many drugs [8].

Many cortical and subcortical parts of the brain, especially in the limbic regions are related to drug-induced neurobehavioral adaptations as well as experience. However, it is confirmed that **t**he most important role in this processes belong to the nucleus accumbens [9].

Golgi morphology of neurons in human nucleus accumbens was poorly described in the available literature, which was the reason to undertake this investigation. The aim of this study was to classify types of human nucleus accumbens. We defined several neuronal types considering their soma size and morphology, dendrite patterns and spine density.

Methods

The present study included 15 adult human brains of both genders, aged 30–65 years. All the brains were taken within 12–18 hours after death. Only normal brains with no visible malformations and without any neuropathological changes or neuropsychiatric history were used. The brains were fixed in phosphate buffered neutral solution of 10% formalin (3.7% formaldehyde) over a period of at least 3 months.

The 20 blocks, originating from 15 brains (30 hemispheres), comprising the septal region were stained according to the Golgi-Kopsch method. The other of the remaining 10 blocks, were cut along the coronal plane and divided into 4 thinner slices. The slices were stained alternately by Nissl and Kluver-Barrera methods in order to enable confirmation of the exact topographical relationships. Application of the Nissl and Kluver-Barrera method was necessary for further delineation of other septal structures and was performed on 10 μm thick coronal sections. The transparency of the Golgi-Kopsch, silver impregnation was the most favorable in 100 μm thick coronal sections without stain precipitations, blood vessels and glia.

We investigated all parts of nucleus accumbens following it rostrocaudally, which merges without a clear border with the medial septal nucleus dorsomedially, and with the basal nucleus and substantia innominata ventrolaterally.

Classification of neurons was performed according to the following criteria: a) shape and size of the cell bodies; b) dendrite organization - the position, number, length and its branching patterns; c) density of the spines covering dendrites; and d) axonal branching patterns. Neurons were drawn using a Camera Lucida Leica DMLB 2 under the magnification of 200× and were photographed under different magnifications.

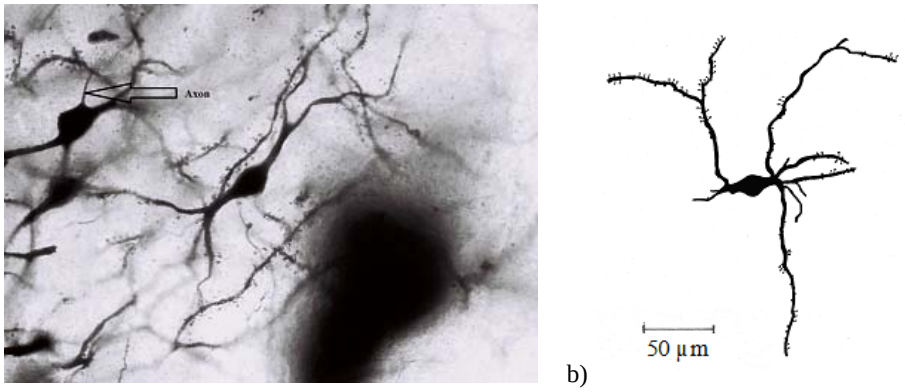
The neuronal soma investigation: maximal length (D max) and maximal width (D min) of perikarya were performed on all of the cells using the Zeiss Axiovision 3.0.6. Aditionally, total dendritic length was controlled by the Sholl analysis [10].

Results

We defined four different types of neurons in human nucleus accumbens, according to their morphology: type I – fusiform neurons; type II – fusiform neurons with lateral dendrite; type III – pyramidal-like neurons, and type IV – multipolar neurons.

Type I – fusiform neuron displayed a fusiform and elongated soma with mostly two long primary dendrites originated from both poles (Figure 1a). The average maximal length (D max) of their elongated perikaryon was 27.99 ± 3.44 μm and the width (D min) was 9.57 ± 0.8μm. The mean total dendrite length (TDL) was 283.34 ± 12.5 μm. Their secondary dendrites were longer than primary ones, but thickness was almost equal (Figure 1b). This frequent neuronal type also predominated in the peripheral region of human nucleus accumbens, the shell, and it was covered by numerous stalked spines, alone its distal dendrites.

Type II – fusiform neuron with lateral dendrite, was found mostly in shell division. In fact, the majority of human nucleus accumbens neurons in the shell division were of this type which exhibited conspicuously featuring on our sections. The specific feature of this type was the constant finding



a) b)

Fig. 1 – Type I (fusiform neuron)

of lateral dendrite arising from the lateral part of the perikaryon (Figures 2, a and b). This thick lateral dendrite was leaving soma mostly from the middle part, under different angles, so we defined as the special type fusiform neurons with lateral dendrite. The perikaryon D max was 26.43 ± 1.22 μm in its long axis, D min was 12.52 ± 0.76 μm wide with 2–3 primary dendrites. Its TDL was 290.98 ± 23.45 μm.

Type III – pyramidal-like neuron, displayed mostly pyramidal or triangular soma, with three major primary dendrite branches. Axons left soma from the part opposite to the strong apical dendrite. Primary dendrites were sparsely covered by the stalked spines, despite the secondary ones with more densely spines (Figures 3, a–f).

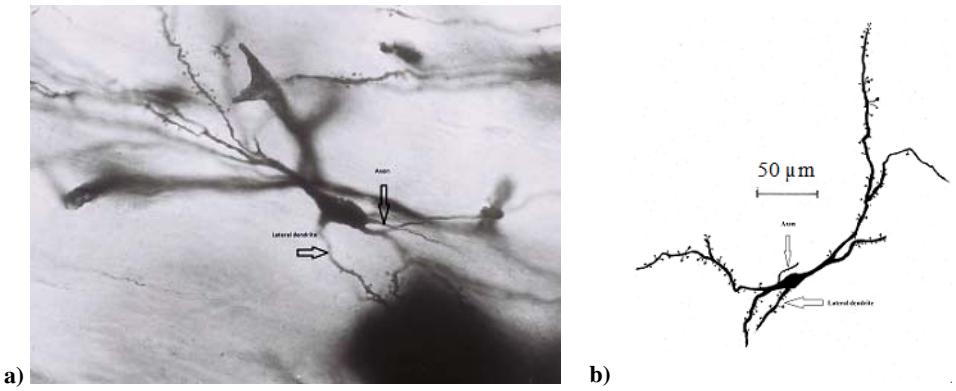


Fig. 2 – Type II (fusiform neuron with lateral dendrite)

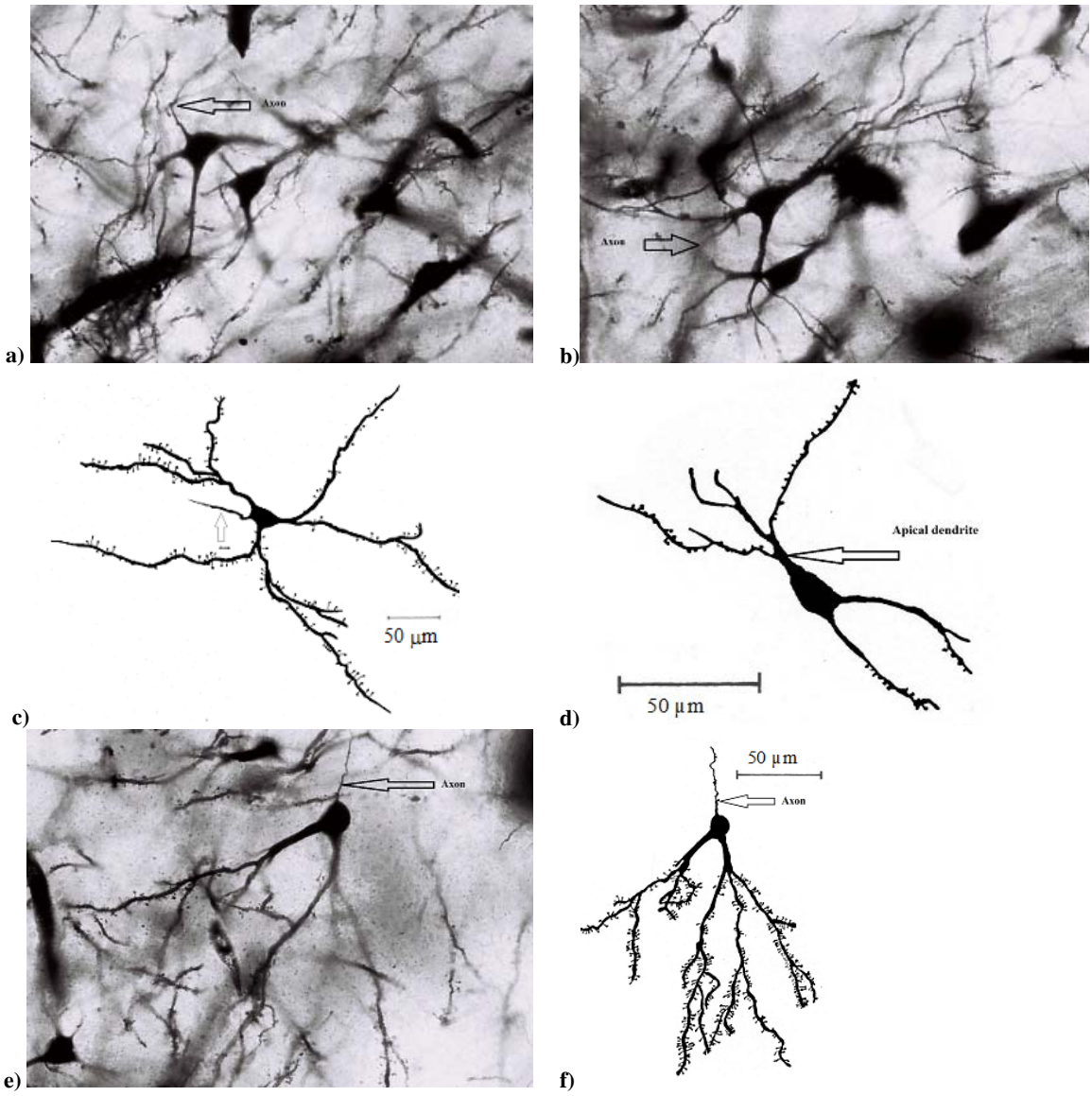


Fig. 3 – Type III (pyramidal-like neuron)

Pyramidal–like neurons had perikaryon D max – 28.3 ± 1.45 μm long, D min was 9.06 ± 0.76 μm wide, and their TDL was 366.02 ± 23.5 μm.

One of the most significant features of these neurons was the presence of the thicker dendrite which raises from the wide base of neuronal soma. Neuronal soma varies from clear pyramidal to elongated pyramidal and piriform. This type was predominant in the core of the nucleus accumbens. Notable was the dominancy of primarily dendrite branch on the "apical" end of neuronal body (Figures 3, e and f). Neurons with piriform soma were sparsely found in shell of the nucleus accumbens with the dense spines on dendrites and occasionally spine like formations on some axons. At the dendrite's end there are many protrusions with the spine clusters at their apex.

Type IV – multipolar neuron, showed different shape of their soma with average six (ranged from 4 to 9) primary dendrites (Figures 4, a–d). The D max of perikaryon was 26.3 ± 1.34 μm and D min was 14.93 ± 2.56 μm. Their numerous dendrites often had varicosities and the primary dendrites were much shorter than the few very long secondary branches which contribute considerably to their mean projected TDL of 354.89 ± 45.3 μm. This neuronal type was found in the core region of the human nucleus accumbens.

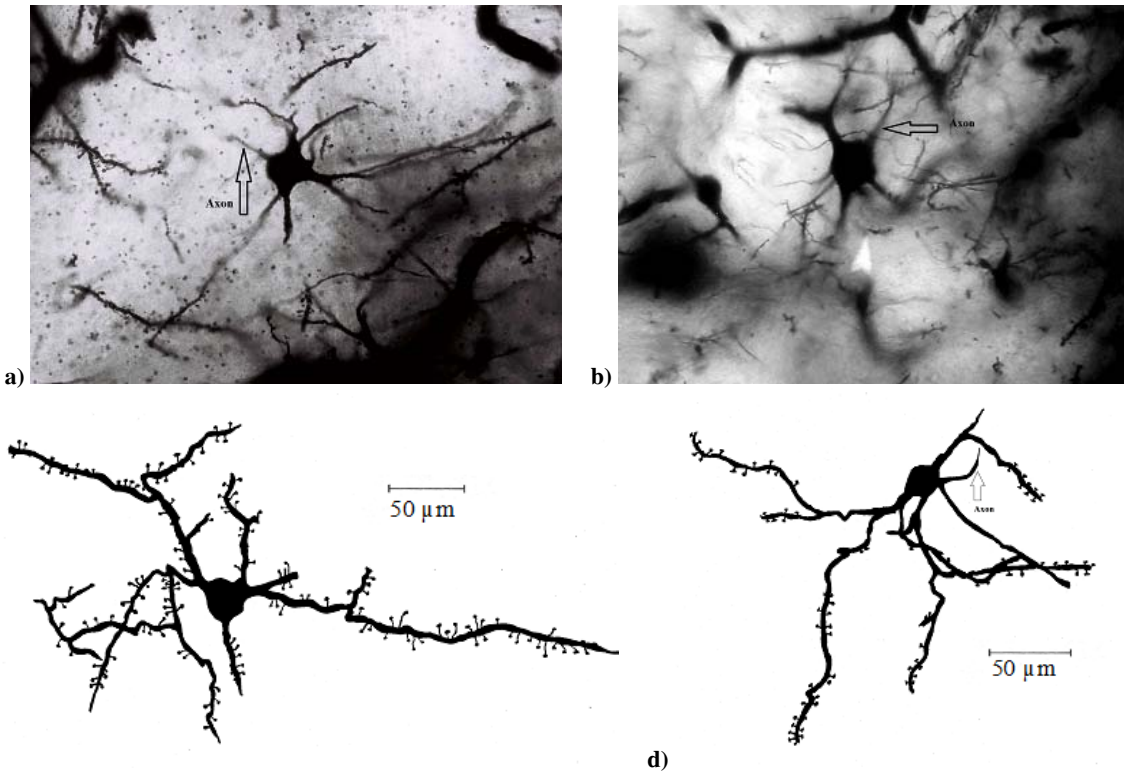


Fig. 4 – Type IV (multipolar neuron)

According to topographical neural distribution we found some difference between the core and the shell. In the shell region of human nucleus accumbens mostly fusiform (type I) and fusiform with lateral dendrite neurons (type II) were predominant, often in groups. Also, their well-arborized dendrite tree was covered by numerous spines. In the core region of human nucleus accumbens, pyramidal-like (type III) neurons were predominant types, but they were usually solitary placed, showing varicose dendrites with stalked spines, and presenting it as medium spiny neurons. Some types of neurons (fusiform, multipolar and piriform) could be classified into larger and smaller subtypes.

Discussion

According to our findings, the border of the human nucleus accumbens to the septum, the shell region, can be recognized by the accumbal fusiform neurons with their longitudinal axis parallel to the convex border to other septal nuclei. The border of human nucleus accumbens to the striatum on Golgi preparations was clearly recognizable by greater cell density and consequent darker appearance of nucleus accumbens. The presented details in the staining sensitivity or selectivity, together with the obvious cytoarchitectural differences and lower impregnation quality of some parts of septal region, may be Golgi-dependent characteristics observed in these regions. It is well-known that the Golgi method is neural and highly selective. On the other hand, this technique provides useful information on the neuronal processes and their branching patterns.

Nucleus accumbens, the shell region, is a part of the limbic region involved in brain functions from motivation and reward to feeding and drug addiction. In the available literature there were few data about Golgi morphology of neurons in human nucleus accumbens. In the cytoarchitectonic study of human nucleus accumbens on Nissl stained sections, Lauer and Hensen [11] mentioned large fusiform, pyramidal- like and granular neurons without detailed descriptions.

We found four major types of neurons in human nucleus accumbens. Fusiform and multipolar types of neurons which we found in human nucleus accumbens correspond to the spiny I type of neurons in monkey striatum described by Di Figlia et al. [12]. Our fusiform neuron (type I) could correspond to the spiny I neurons with flattened soma described by some authors [12]. However, human striate spiny neurons with six primary dendrites described by others [13] could correspond to our multipolar neurons. Our pyramidal-like neurons correspond to spiny type II of Di Figlia et al. [12] with about smaller spine density than spiny I type. Both spiny types (I and II), were considered as the efferent elements, but we did not find aspiny types also described in monkey striatum in human nucleus accumbens [12, 13]. Our finding of type IV, multipolar neuron (Figures 11 and 12), corresponds to medium spiny neurons described by other authors. Medium spiny neurons consist of 2–6 primary dendrites, different thickness, with dense spines on secondary and third dendrite branches [14–17].

We compared the morphology of rat nucleus accumbens neurons and its projection to the substantia nigra, examined by Meredith et al. [6], with our results. Their findings were different in significantly larger number of the spines in the core than in the shell region, similar morphology of perikaryons in both regions of nucleus accumbens (round to oval perikaryons), and the smaller size of perikaryon in the rat (equivalent diameter 9–15 μm). On the other hand, their findings of densely spine dendrites, less primary dendrites than secondary and core dendrites branching under the sharp angle were similar to ours. Possible differences can be attributed to the smaller, different and very specific sample of their neuronal population in rat nucleus accumbens [18, 19].

In mammalian brain psychostimulant drugs induce changes of brain plasticity such as altering dendrite branching, spine density and/or density and synaptic organisations in medium spiny neurons in the nucleus accumbens. Repeated exposure to some psychostimulant drugs is related to behavioural and long lasting changes in the brain. Some of them are transitory and others are persistent, resulting in irreversible changes in the neural structure and behaviour. Thus, well established knowledge of neural morphology is essential for understanding the drug related changes [20–24].

Despite many investigations of medium spiny neurons that exhibit morphological alteration in usage of psychoactive drugs [25–28], their neuroanatomical characteristics remain unexamined, what requires more detailed analysis.

Lower density of impregnated neurons in the core region of the human nucleus accumbens and their higher density in the shell region, with the fusiform and pyramidal-like type neurons (neuronal types most rich in the spines) which we found as the predominant types in shell region, suggest probably nucleus expansion, during the human phylogenesis, favoring the limbic functions.

Conclusion

Because of the great motivational, emotional and other limbic demands, as like as the limbic expression in humans, our results indicate a great morphological complexity of human nucleus accumbens neurons, as it was expected. In general, our findings point to the functional importance of human nucleus accumbens, as an important region for emotional and motivation processes in basal forebrain [28–30].

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Received on February 22, 2010.
Revised on March 19, 2010.
Accepted on April 21, 2010.