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Neuronal morphology of dorsal cerebral cortex of the Indian wall lizard, *H. flaviviridis* (Rüppell)

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Abstract : The cerebral hemisphere of lizards is divided into pallium (cerebral cortex) and subpallium. The structural organization of dorsal cerebral cortex of the *H. flaviviridis* has been studied with the help of neurohistological techniques. The dorsal cortex shows three neuronal layers viz. outer plexiform layer, middle cellular layer and inner plexiform layer. Using characteristics such as criteria of location, soma shape and size, dendritic tree pattern/dendritic field shape, and dendritic spine covering four types of neurons have been distinguished in the cellular layer of dorsal cortex: monotufted neurons (18.14 %), bitufted neurons (28.43 %), multipolar neurons (22.55 %) and pyramidal neurons (30.88 %). The comparative study of the morphology of these neurons represents their similarities with other animal groups.

Key words: lateral cortex /bitufted neuron/ spinous/Golgi study/lizards.

Introduction :

The reptiles are interesting class of vertebrates in many respects and exhibit the highest degree of diversification because they were the first vertebrates to inhabit the terrestrial mode of life. Consequently a lot of physiological, morphological and behavioral changes have occurred in them including the modifications in brain. The cerebral cortex of lizards represent a laminar structure in which most of the neuronal cell bodies are grouped forming a principal cell layer sandwiched between the inner and outer plexiform layers (Luis de la Iglesia & Lopez-Garcia, 1997; Lopez-Garcia *et al*., 2002; Srivastava *et al*., 2007, Maurya and Srivastava, 2006; Srivastava *et al*., 2009; Srivastava and Maurya, 2009a, 2009b; Maurya *et al*., 2011), which are populated by scarce interneurons and where the afferent connections terminate in a highly laminated fashion (Lopez Garcia *et al.*, 2002).

By using Golgi-impregnation method different workers described various numbers of the neuronal types in all the four cortical areas of the lizards (Srivastava *et al*., 2012). In the medial cortex only one type of neurons in snake genera *Natrix* and *Boa* (Ulinski, 1977), five types in the lizard *L. pityusensis* (Berbel *et al.*, 1987), *P. hispanica* (Luis de la Iglesia & Lopez-Garcia, 1997), *M. carinata* (Srivastava *et al.*, 2007) and seven types in *H. flaviviridis* (Maurya and Srivastava, 2006) have been reported. In case of dorsomedial cortex one type of neurons in the lizard *A. agama* (Wouterlood, 1981), three types in each layer of snake's dorsomedial cortex (Ulinski, 1979), six types in *H. flaviviridis* (Srivastava *et al.*, 2007) and seven types in *C. versicolor* (Sakal *et al.*, 2010) have been reported. In the dorsal cortex four types of neurons in the lizard *P. algirus* (Guirado *et al.*, 1987), five types in *M. carinata* (Srivastava *et al.*, 2007) and seven types in *C. versicolor* (Sakal et al., 2010) have been reported. In lateral cortex four types of neurons are present in all the three layers of snakes (Ulinski and Rainey 1980), three types were observed in the *M. carinata* (Srivastava *et al*., 2007) and *C. versicolor* (Sakal *et al.*, 2010).

Since, there exists a wide variation in the composition of different parts of brain in different reptilian orders and also among various species of lizards due to behavioural and environmental factors. Hence, in order to increase our knowledge the present study provides the morphology of the neurons of the dorsal cerebral cortex of the telencephalon of the Indian wall lizard, *H. flaviviridis.*

Materials & Methods :

A total of 32 male lizards (*Hemidactylus flaviviridis*) were used in the present study (5 for Nissl staining and 27 for Golgi study). The adult lizards with 60 – 85 mm snout to vent size have been taken for the experiment. Animals were captured from the surroundings of Allahabad (Uttar Pradesh, India), and kept in terrarium prior to the experiments. Five anaesthetized adult lizards (*H. flaviviridis*) were perfused with 100 ml of physiological saline followed by 10% formalin solution for 1 hour. The brain was immediately removed out from the skull and fixed in 10% formalin. The brain was processed for Cresyl violet staining to study the Cyto-architecture of the cerebral cortex of the lizard (Srivastava *et al*., 2009). Under ether anesthesia, animals were perfused with 1% paraformaldehyde and 1.25% glutaraldehyde in 0.1M phosphate buffer, pH 7.2-7.4 by immersion in the fixative. The brain was immediately removed out from the skull and kept overnight in the same fixative at 4°C. For staining, the Golgi-colonnier method was used (Colonnier, 1964) with some improvements (Luis de la Iglesia and Lopez-Garcia, 1997) adapted to the present material (i.e., 3 to 5 days of indurations at 4°C in a mixture of 2.4% potassium dichromate and 5% glutaraldehyde followed by 1 to 2 days of impregnation in 0.75% silver nitrate). After impregnation the brain was dehydrated in different grades of alcohol, cleared in xylene and embedded in paraffin wax (m. p. 52-56°C). 60 to 100 µm thick transverse sections were cut by the rotatory microtome. Sections were cleared in xylene and mounted in DPX. Photomicrographs were taken with computer aided microscope, Nikon eclipse 80i (Software, ACT-1).

Results :

The cerebral hemisphere of *Hemidactylus flaviviridis*, is composed of a roof (pallium) and a floor (subpallium). In the pallium (cerebral cortex) four cortical regions were present medial cortex (MCx), dorsomedial cortex (DMCx), dorsal cortex (DCx) and lateral cortex (LCx) (Fig. 1A). The septal area occupied the medial portion of the subpallium, whereas the lateral portion organized by the dorsal ventricular ridge (DVR) and the striatum (Str).

The dorsal cortex of *Hemidactylus flaviviridis* displayed three distinct neuronal layers and an ependymal layer. The basic pattern of three layers: outer plexiform layer (opl), cellular layer (cl) and inner plexiform layer (ipl) could be seen in the cresyl violet stained transverse sections through the cerebral hemisphere. The thickness of outermost layer-I ranged from 89 – 200 µm. Layer-II was characterized by densely packed neuronal cell bodies. The thickness of layer-II ranged from 61 – 93 µm. Layer-III was 65 - 104 µm thick having loosely packed neuronal cell bodies (Fig. 1B).

The four cortical regions of cerebral cortex were not continuous with each other and the sheet of somas in the cell layer-II was interrupted by a discontinuity from rostral to caudal regions. The dorsomedial cortex was overlapped with the medial extreme of the dorsal cortex whereas the lateral portion of the dorsal cortex overlapped by the lateral cortex (Fig. 1A). The result of this discontinuity and overlap is that the basic trilaminar pattern have been replaced by five-layered cortex within the annulus of overlap.

A Golgi analysis of the cell types present in the dorsal cerebral cortex of the lizard *Hemidactylus flaviviridis* gave highly variable results depending on individual animals or unknown experimental factors. The study of dorsal cortex has been carried out in the sections, in which the maximum number of impregnated neurons was achieved. The neurons were carefully selected for the study that showed well-developed dendritic tree pattern and clear dendritic branching. The axonal branching pattern was also traced for exploring the connections.

Using characteristics such as criteria of location, soma shape and size, dendritic tree pattern/dendritic field shape, and dendritic spine covering, four types (Table 1) of neurons have been distinguished in the cellular layer of dorsal cortex: monotufted, bitufted, multipolar and pyramidal neurons.

Monotufted neurons :

The monotufted neurons are of medium size and located at the upper border of the cellular layer. Their cell bodies are spherical or polygonal shape and measures on average 15 µm × 10 µm. The upper half of the soma gives rise to one or two primary dendrites, which usually gives off secondary dendritic branches in the outer plexiform layer (Fig. 1C). These dendrites are smooth or covered by few sparsely distributed spines on their surface (Fig. 2A). The dendrites arise from apical part, go directly towards the pial surface and then run parallel to the pial surface in the outer plexiform layer. The length of the dendrites of these neurons ranges from 75 µm to 110 µm. Axon and axon collaterals are not observed in these types of neurons in the present study.

Bitufted neurons :

The bitufted neurons are usually located in the

Table 1. Characteristic features of the projection neurons of the dorsal cerebral cortex of the lizard, *H. flaviviridis*.

Type	Descriptive name	Layer	n	%age	Mean soma size (µm)
1.	Monotufted neurons	cl	37	18.14 %	15 × 10 µm
2.	Bitufted neurons	cl	58	28.43 %	15 × 10 µm
3.	Multipolar neurons	cl	46	22.55 %	16 × 10 µm
4.	Pyramidal neurons	cl	63	30.88 %	16 × 11 µm

Percentage is given as n.100/204, 204 is the number of neurons examined in this study.

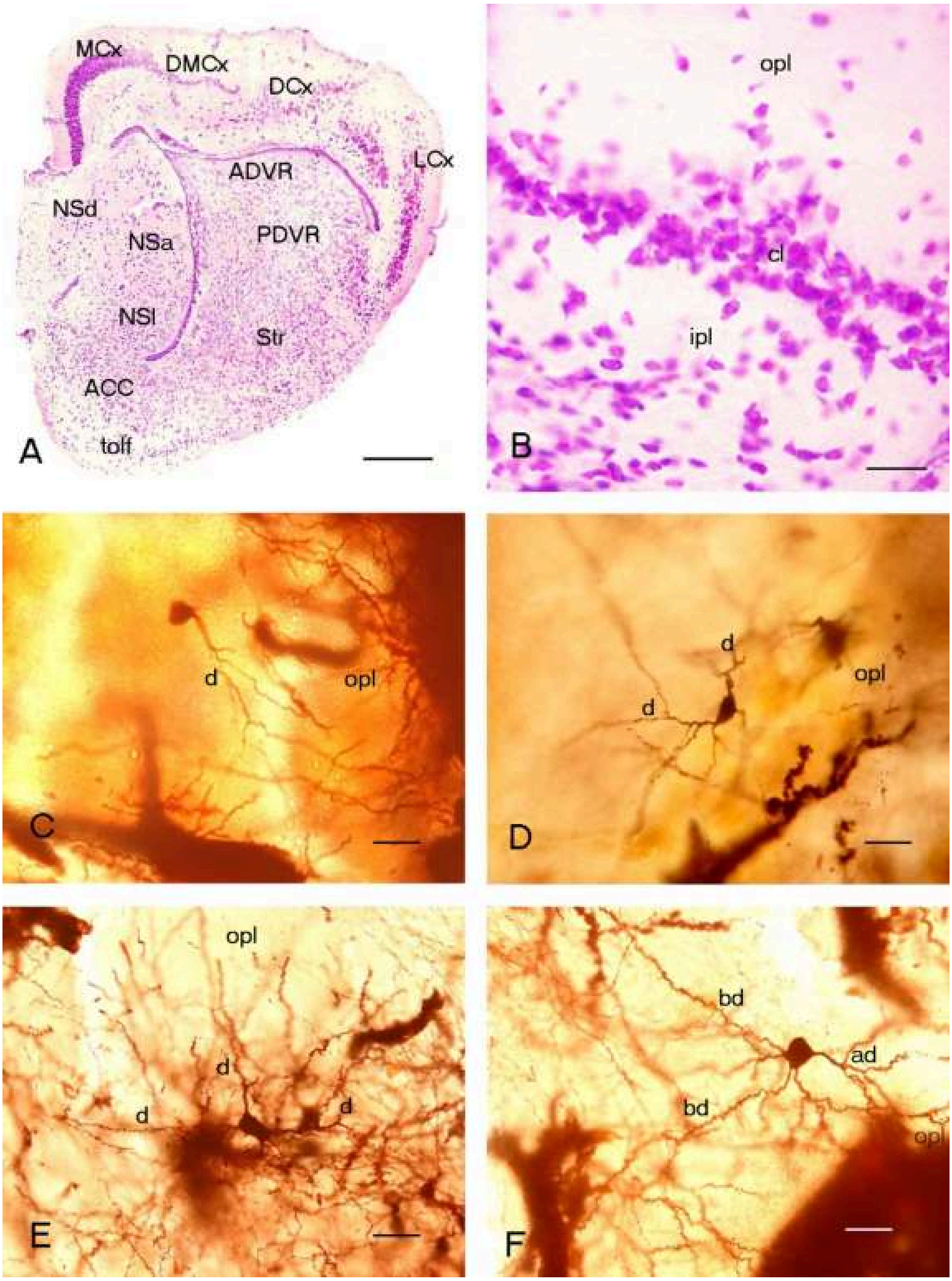

Fig. 1. (A) Cytoarchitecture of the *H. flaviviridis* cerebral cortex. (B) Nissl stained section of dorsal cerebral cortex. Neuronal types in the dorsal cerebral cortex of *H. flaviviridis*: monotufted (C), bitufted (D), multipolar (E), and pyramidal (F). Bars 100µm (A), 25µm (B-F). ADVR – Anterior dorso ventricular region, MCx – Medial cortex, DMCx – Dorsomedial cortex, DCx – Dorsal cortex, V – Ventricle, cl – cell layer, SE – Septum, LCx – Lateral cortex, opl – outrér plexiform layer, ipl – inner plexiform layer, d- dendrite, ad- apical dendrite, bd- basal dendrite, cl- cell layer.

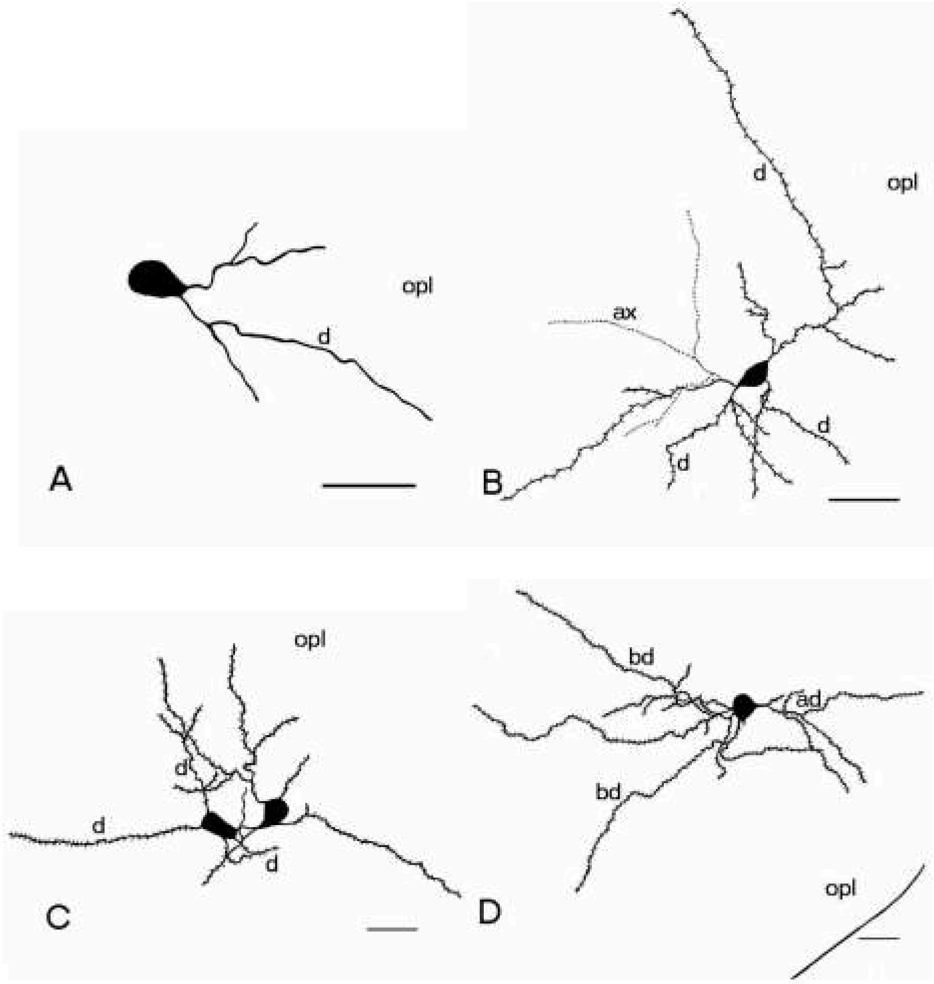

Fig. 2. Camera lucida drawing of the dorsal cerebral cortex neurons: monotufted (A), bitufted (B), multipolar (C), and pyramidal (D). Bars 25µm (A-D). opl- outer plexiform layer, d- dendrite, ad- apical dendrite, bd- basal dendrite, ax- axon

middle region of the cellular layer. These neurons have somata of elongated or superficial shape which measures on average 15 µm × 10 µm. Primary apical and basal dendrites arise from the two poles of the soma and, after extending for only short distances, they branched to produce apical and basal dendritic tufts (Fig. 1D). The dendritic branches of these neurons have spinous covering on their surface. The apical dendritic tuft, which extends towards the outer plexiform layer, is larger than the basal dendritic tuft (Fig. 2B). The dendritic extent of apical tuft ranges from 64 µm to 169 µm, while the extension of the basal dendritic tuft ranges from 36 µm to 119 µm. The axon usually arises from the basal pole of the soma or from a basal dendrite. It enters to the inner plexiform layer and run parallel to the ependymal layer. It gives off a number of thin collaterals, some of which curved back towards the cell body. The range of axon extension is 41 µm to 82 µm.

Multipolar neurons:

The multipolar neurons have oval or rectangular cell body present in the cellular layer. The somata measures on average 16 µm × 10 µm. The spinous dendrites are originated from the cell body and radiates in all direction towards the outer and inner plexiform layers (Fig. 1E). The length of dendrites in these neurons ranges from 32 µm to 151 µm. The mean length of dendrites is 67.8 µm. The axon of these neurons arises from the upper half of the cell body and reaches the outer plexiform layer (Fig. 2C). The axon length in these neurons ranges from 96 µm to 164 µm.

Pyramidal neurons :

These neurons have conical to pear shape cell body, on average 16 µm × 11 µm. From the apices of these neurons arise one or two smooth primary apical dendrites, branching in the secondary and tertiary dendrites in the outer plexiform layer. These secondary and tertiary dendrites normally reach the pial surface and bear many spines (Fig. 1F). The length of apical dendrites ranges from 82 µm to 171 µm. The pyramidal neurons have few primary dendrites emerging from the lower part of the cell body and extending their secondary branches in the inner plexiform layer forming the basal dendritic tuft. The length of basal dendrites ranges from 69 µm to 164 µm. The axon of these neurons arises from the basal pole of the cell body and bifurcates near the soma usually emitting two branches (Fig. 2D). These branches enter the inner plexiform layer where it gives off collaterals, which run parallel to the inner plexiform layer. The axon length ranges from the 69 µm to 124 µm.

Discussion :

Dorsal cortex was present on the dorsolateral surface of the cerebral hemisphere in all snakes and lizards. In the lizard *Hemidactylus flaviviridis* the dorsal cortex was present at the dorsolateral surface of the pallium and consist of three layers viz. from the pia to the ependyma, a superficial plexiform layer, a cellular layer and a deep plexiform layer. It could be divisible into two subdivisions, the dorsal cortex medialis and dorsal cortex lateralis. The medial and lateral edges of the dorsal cortex overlaped with the dorsomedial and lateral cortices giving rise to the “superposito medialis” and “superposito lateralis” respectively. In snakes such as *Eryx* and in turtles (Northcutt, 1970; Platel *et al.*, 1973) little overlapping was observed while crocodilians show no overlapping (Crosby, 1917; Platel *et al*., 1973). Thus the five layered pattern at the edges due to overlapping is a lacertilian character. The dorsal cortex of the lizards *Iguana* (Northcutt, 1967) and *Psammodromus algirus* (Guirado *et al*., 1987) had been divided into two subdivisions medialis and lateralis. The subdivisions in the dorsal cortex of *M. carinata* had not been observed (Srivastava *et al*., 2007) which shows its less developed condition.

Using various characteristics four types of neurons in the cellular layer of *H. flaviviridis* namely monotufted, bitufted, multipolar and pyramidal neurons have been described. In the *Lacerta galloti*, while considering the size and location within the dorsal cortex only single type of neuron had been described in the granular stratum (Garcia-Verdugo *et al.*, 1983). This neuron showed large neuronal somata which received axo-somatic synapses and covered by ependymoglial ramifications. This neuron represents 60% of all somata of the dorsal cortex observed in the *Lacerta galloti* (Garcia-Verdugo *et al.*, 1983) and seven types have been observed in *C. versicolor* (Sakal *et al.*, 2010). The additional criteria such as dendritic tree pattern and spine covering allowed to describe three types of neurons namely bitufted, multipolar and pyramidal neurons in the lizard, *Psammodromus algirus* (Guirado *et al.*, 1987). Five types’ monotufted, bitufted, candelabra, pyramidal and bipyramidal neurons have been differentiated in the cellular layer of dorsal cortex in *M. carinata* (Srivastava et al., 2007). Recently De Carvalho Pimentel et al. (2011) described ten neuronal types in the dorsal cortex of the lizard *Tropidurus hispidus.* Most of these neuronal somata were dispersed without forming a conspicuous cell layer. Thus the classification criteria used in considering the types of neurons may play a major role. Due to this reason two or more previously reported types match with the single type in latter studies or vice versa. In addition difference in the morphology of the neurons between the different species can be expected since there is a considerable variation between them.

All the neurons of the dorsal cortex cell layer of *H. flaviviridis* showed homologous large size somata averaging 15 x 10 µm. This observation resembled to that of large neuronal somata (type B) of granular stratum of the dorsal cortex of *Lacerta galloti* (Garcia Verdugo *et al*., 1983). The projection neurons of the cell layer of dorsal cortex in *H. flaviviridis* were found to be uniformly distributed. The monotufted neurons observed in the *H. flaviviridis* had not been reported in any lizard except in *M. carinata* (Srivastava *et al.*, 2007). The bitufted neurons, multipolar neurons and pyramidal neurons observed in the *H. flaviviridis* have clear matching types reported in the *Psammodromus algirus* (Guirado *et al.*, 1987), *M. carinata* (Srivastava *et al.*, 2007) and *C. versicolor* (Sakal *et al*., 2010) on the basis of their similar morphology.

Anatomically, the three neurons of reptilian dorsal cortex namely bitufted neurons, multipolar neurons and pyramidal neurons have formed the basis for comparing the medial aspect of dorsal cortex being the homologous to the mammalian hippocampal formation (Lacey, 1978; Guirado et al., 1998), whereas the lateral aspect of the dorsal cortex had been compared to the mammalian isocortex, or at least to part of it (Ulinski, 1990; Ten Donkeelar, 1998). These three neurons are found to be dominant neuronal types in both reptiles and mammals. The dorsal cortex is a structure unique to reptiles, and its relationship to structures in mammalian brain is of great theoretical interest such as the dorsal cortex of lizards differ from the isocortex in being three layered instead of six layered and in lacking columnar organization (Reiner, 1993).

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