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Intermediate cells of Lugaro in the cat cerebellar cortex

A.B. Melik-Moussian, V.V.Fanardjian

L.A.Orbeli Institute of Physiology, National Academy of Sciences of Armenia

22 Orbeli Bros. Str. 375028, Yerevan Republic of Armenia

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Despite over a century's comprehensive study of cerebellum till this day "white spots" are revealed in its structural organization. Foundation of cerebellar structure was layed by Cajal now confirmed by electron microscopic investigations. Nevertheless, the classical histology considered now as exhausted itself and the new morphological structure of cerebellum can be introduced by a modern direction in morphology – immunochemistry opening broad possibilities in studying the important aspects of plasticity of cerebellum and its function in motor control and operantly memory.

In literature, however, since the 90s some interesting information has been appearing regarding the structural and ultrastructural organization of mammalian cerebellum obtained by using the classical histology technique. Thus new monodendritic neurons resembling paintbrush – "brush cells" have been discovered in granular layer of cerebellar cortex especially in "vestibulo-cerebellum" [15].

Initially candelabrum cells are described as new independent interneurons arranged as a sandwiches between the soma of Purkinje cells with their specific axonal arborization in molecular layer [10]. New data have appeared in relation to the parallel fibers [8] as the important component in cortical structure of cerebellum, and their influences on the function of Purkinje cells.

It has been clarified that localization of synapses of the same axon, namely the axon of granular cell on different parts of dendritic arborization of Purkinje cells, plays different roles in their function.

It is well known that the granular cells compose 90% of the granular layer. Rising vertically to the periphery of cerebellar cortex through the granular layer, the axons of the granular cells develop numerous synapses on their ascending segments with distal dendrites of the second division of Purkinje cells. The axons of granular cells are arranged densely at the level of Purkinje cells in the beginning of the molecular layer. Here they form 56% of such synapses which after dividing in a T-form compose a net of parallel fibers with the diameter 1,2-1,3 μm. On this fact for now nobody has payed any attention except Gundappa-Sulur et al. [8]. It has been considered that all axons of granular cells after entering the molecular layer are divided as antennas contacting in all thickness of the molecular layer with dendrites of Purkinje cells; more superficially located granular cells near the layer of Purkinje cells send their axons to the more peripheral part of molecular layer, in the direction of pia mater. Here the distribution of parallel fibers is not so thick in density as in the molecular layer with diameter – 5,2 μm [20]. Besides, the contacts between ascending segments of axon of the granular cells and dendrites of Purkinje cell reach only 8% [8]. That means that the axons of granular cells contacting with different parts of dendritic arborization of Purkinje cells have a quantitatively and qualitatively different influence on their function. Some of them contact with Purkinje cells dendrites through their spines with intermedial parts of II and III dendrites division and with the large dendritic trunks. Due to such arrangement these parallel fibers are responsible for the behavior of Purkinje cells dendrites. The second type of synapses of ascending segments, mainly located at the beginning of molecular layer, are responsible for the function of their soma [8].Thus, two types of synapses formed by the axons of granular cells differ from each other morphologically as well as functionally.

The study of localization of NO /nitric oxide/ in Lugaro cells by the reaction of diaferase [16] has demonstrated that despite high level of enzyme activity in granular cells of hemispherical parts of cerebellar cortex, diaferase negative granular cells have been revealed.

The data obtained in a series of investigations of structural elements plasticity of cerebellum [22] have shown the main excitatory input-climbing fibers playing an important role in maturation and development of Purkinje cell dendritic tree arborization and put up the problem of degeneration with consequent regeneration of nervous elements in plasticity of cerebellum on a new level. However, the complicated function of cerebellum is mainly adjusted only by Purkinje cells.

Material and Methods

The experiments were performed on adult young cats and fortnight kittens in accordance with the Principles of Laboratory Animal Care Guide (NIH Publication 85-23, revised 1985), and were approved by the Institutional Animal Care and Use Committee. All efforts were made to minimize animals' suffering and to reduce the number of animals used. After deep anaesthesia (55 *mg/kg* mass of animals) the brain was removed. The adult cat was perfused through the heart 3,5% solution of bichromate potassium on the 10% neutral formalin. The kittens were choked under the "ether pro narcosis". In all experiments the silver technique impregnation by Golgi-Kopsh was used. The cerebellum was cut under the 100-120 *mμ* in three directions: sagittal, frontal, horizontal. Camera lucida RA- 4 was used for reconstruction of neurons.

Results and their Discussion

It is known that criteria of neurons types are: the form of cellular soma, its localization, dendritic ramification and axonal arborization. Accordingly the intermedial Lugaro cells represented in granular layer are of two types:

1. spindle-shaped or fusiform cells located horizontally, vertically or at the angle 60-70° to the longitudinal folial axis, and

2. of remarkably precise geometrical form – triangular neurons.

Both of them have no specific disposition as "brush" neurons and are situated in the whole cerebellar cortex. Our observations have shown their localization which is at the top of the folium or directly as a part of it.

Initially these spindle-shaped spherical or polygonal forms of neurons at the end of the last century were described by Golgi [7], later more in detail by Lugaro [11,12] and then by Cajal [2]. Further studies were performed on monkeys [5], rats [1,17,19] and rabbits [16]. The fusiform Lugaro cells located directly under the Purkinje cells are more known in literature, although we have observed this type of horizontal cells at different levels of the granular layer. Not all of them are real Lugaro cells. These neurons as Lugaro ones have a soma with polar output of dendrites, one from each side, smoothly moving away from cell body along 200-300 *μm* and more. Sometimes they have a vertical direction and pass perpendicularly to the axis of the folium or obliquely to it with a diagonal direction of polar dendrites. According to the position of their soma the direction of their processes corresponds; under the vertical position of cellular soma dendrites oriented up and down, under the horizontal position–right and left. Some of them are located at the top of the folium and have dendrites moving away from both poles, approximately at an identical distance from cell body. The dendrites are divided into dendrites of I, II, III order, which have a little winding at the end. Except the main dendrites, these neurons in deep parts of granular layer gave 1-2 thin and long recurrent brunches rising from soma or primary dendrites. The latter were usually smooth and had needle-shaped or short bulb-like rare spines located frequently at the distal parts of the dendritic trees. Axons of fusiform cells could not only move away from the cellular soma, but also from dendrites of I, II, III order.

We distinguished several ramifications of axons among fusiform cells:

1. The axons of fusiform cells following the molecular layer, giving collaterals reaching the level of dendrites of Purkinje cells in molecular layer. Sometimes they can be transferred to another side of the folium but never out of it.

2. The fusiform vertically distributed cells sending their axonal collaterals to the molecular as well as the granular layer.

3. The fusiform Lugaro cells, lacated horizontally under the layer of Purkinje cells, directing their axons along the layer of Purkinje cells, providing collaterals to the whole row of Purkinje cells [13,14], giving few branches to the molecular and granular layers. Powerful influence was confirmed by the contact of one Lugaro cell with several Purkinje cells in the ganglionar layer in proportion 1:18-25 [8,13,14,21].

Such distribution of axonal ramification in relation to Purkinje cells layer is confirmed by other investigations [10,16,21]. The observations have shown that independent of localization of Lugaro cell's body at different levels of the granular layer departure of its processes as a rule remains in folium.

4. Located in the thickness of granular layer the axons of some Lugaro cells gave collaterals which never reached up the level of the molecular layer, branching only in the granular layer without any output.

Naturally they cannot participate in the inhibitory action. Evidently there are namely neurons doubtfully by Fox [5]. Are all the fusiform cells real Lugaro neurons?

The triangular Lugaro neuron described by Lugaro as a triangular cell with short dendrites [11,12], as a fusiform one, has branches in vertical and horizontal directions. In this position (in our material) the triangular cells are directed parallelly or perpendicularly to the axis of folium (correspondingly the processes). Thick single dendrite outputs from the top of triangular cell for 40-50 μm follow division on II and III orders, composing rather narrow plexus of dendritic ramification with a little winging at the end. These neurons resemble a little neurons of central cerebellar nuclei with their "tufted"ends. However, as distinct from them, they have longer polar primary dendrites, dividing on the II and III order, reaching sometimes for a long distance from cell body about 425 *μm*, weekly ramified along their way. The axon of the triangular neuron rising from one of ths side or at the top of it, passes a short distance and disappeares in surrounding tissue. It is known that they are hardly impregnated [15]. However, it is necessary to state that in none of the investigated cases axons with their collaterals from outer cerebellar cortex are lither fusiform or triangular neurons. They can pass from one side of folium to another but never leave it. On the contrary, dendrites have rather long extent and reach the white matter itself or neighboring folia.

Owing to the distribution of cell body and departure of their axon, Lugaro cells have a great space extent: from the molecular layer through axon's collaterals till its own or neighboring folia white matter, composing favorable conditions for numerous contacts with a number of neuronal elements of the cortex. These contacts are formed by the cell body, axon, dendrites of I,II,III order and can be both axosomatic and axo-dendritic. They can terminate on the cell body and dendrites (proximal or distal) or simultaneously on the soma and dendrites composing polysynaptic contacts. Such kinds of contacts are produced by basket cells, parallel fibers, mossy fibers with soma and primary dendrites of Golgi neurons, with clawing dendritic ending of granular cells, with Purkinje cells and dendrioles and soma brush neurons, axon terminals of brush neurons on the dendrites of Lugaro cells.

In our material we often can see "clusters" of several Lugaro cells (2-3), contacting with the neuronal elements of cerebellar cortex, suggesting the synaptic transmission between them. This was confirmed by other investigators [16].

Besides 8 types of neurons, some neurons resembling Lugaro neuron are present in the cerebellar cortex. These neurons are possibly those described by Fox [5]. These cells by their appearance are similar to Lugaro cell, but differ from it because of dendritic departure from soma. The classical Lugaro cells have smoothly polar dendrites at each opposite side of soma and are divided to the I,II,III order ; it is very difficult to define the beginning of primary dendrite from cell body. Not really Lugaro cell can departure its primary dendrite from oval soma with an acutely marked of it . So, it is possible to suggest that Lugaro cells form contacts with almost all neuronal elements of the cortex distributed at different levels of the granular layer and have a different direction of cell's body in the granular layer.

One of the remarkable peculiarities of Lugaro cells is their flat distribution of dendrite arbor in distinction to the vertical direction of the rest of interneurons of cerebellar cortex.

It is more interesting to present the "X" form of neurons in cerebellar cortex with central situated cell body and divergent dendrites from it, the arborization of which takes horizontal position in the plane of folium.

On the foundation of our account at present 8 types of neurons with different morphological characteristics, distinct functional purposes in three-layer cortex of mammalian cerebellum are discovered. All these 8 types of neurons are identified by their localization, form, size of cell body, dendritic ramification and axonal arborization. They are distributed as follows: in the molecular layer–star cells, basket neurons, and recently discovered candelabrum neurons; in granular the layer – the row of Purkinje cells – the Purkinje cell layer, and in the granular layer – granular cells with specific arrangement of their synapses on the dendrite of Purkinje cells, Golgi neurons, and less investigated Lugaro cells and brush neurons. From all the pointed out neurons particularly interesting are the interneurons of three types: brush cells, caudelabrum neurons of lower parts the of molecular layer and intermediate cells of Lugaro, about physiological significance of which important new data have appeared.

The immunohistochemical study and single cell reconstruction of the Lugaro cell show that Lugaro cell axons form a parasagittal plexus and by longitudinal axons extend along to the parallel fibers and contact with Golgi cells. The Lugaro cell synapses are located at the basis of the Golgi cells apical dendrites. In the cerebellum a single Lugaro cell contacts > 100 Golgi cells. The Lugaro cells are selectively excited by serotonin, in contrast to stellate, basket and Golgi cells. The serotonin increases the frequency of IPSCs in Golgi cells made Lugaro cells as the source of serotonin-driven inhibition of Golgi cells. The Lugaro cells are excited by serotonin at the same concentration that evoked IPSCs in Golgi cells and the frequency of firing of the Lugaro cells is the same range as the frequency of serotonin evoked IPSCs in Golgi cells [3].

The Golgi cells receive both pure GABAergic inhibition from the basket and stellate cells and mixed GABAergic and glycinergic inhibition from the Lugaro cells [4]. GABA is the predominant inhibitory neurotransmitter in the cerebellum [6], but glycin is also found in inhibitory interneurons of the granular cell layer and in fibers of the molecular layer [18,25]. GABAergic and glycinergic neurotransmitters co-exist in many structures of the central nervous system. Co-localization of the two neurotrasmitters is prominent in the spinal cord [23,26] and others sensory and motor centers [18,24].

We suppose that it is impossible to place stellate and basket cells in a single group, although both of them are present in the molecular layer. They are distinguished from each other not only topographically, morphologically and functionally, but by the contacts with Purkinje cells, Lugaro cells and the other neuronal elements of cerebellar cortex [10,16,21].

The electron microscopic study of the synapses has revealed on the ascending segments of the axons granular cells differing by caliber, quantity of synaptic vesicles, their size, intensity of color and numerous from the synapses of parallel fibers – spines of dendrites of Purkinje cells. The density of their distribution and caliber of the fibers in the peripheral parts of the molecular layer are changed (5.2 μm at the periphery and 1,2-1,3 μm at the foundation of the molecular layer) meanwhile the latter more than the former [20]. So, on the basis of our material and literature data two types of Lugaro cells–fusiform and triangular in cerebellar cortex of the cat are presented. Their axons never leave the cerebellar cortex and therefore can be considered as interneurons; as they contain GABA and glycinergic fibers of these interneurons they can be counted as interneurons of inhibitory type.

Taking into account the functional capabilities of Lugaro cells we think that Purkinje cells receive huge inhibitory input only from Lugaro cells from three directions:

1. From the axon collaterals of Lugaro cells branching in the molecular layer till the level of dendritic arborization of Purkinje cells;
2. From the axon collaterals of Lugaro cells giving branches not only in the molecular layer, but 1–2 short branches in the granular layer too;
3. From the huge axonal arborization of Lugaro cells through the row of 18-25 Purkinje cells following along their way in the ganglionic layer of Purkinje cells;
4. From the ascending segments of axon of the granular cells, forming synapses with the second order of the dendrites of Purkinje cells just near the soma of Purkinje cells;
5. From the synapses of parallel fibers – Purkinje cell spines in the thickness of the molecular layer responsible for the behaviour of the dendrites of Purkinje cells;
6. From the descending axon collaterals of basket cells at the beginning of the axon of Purkinje cell;
7. From the descending axon collaterals of busket cells contacting with the dendrites of I, II, III orders of Lugaro cells;
8. From the descending axon collaterals of busket cells through the polysynaptic contact;
9. From the stellate neurons of molecular layer cell body and dendrites of Lugaro cells;
10. Through the Lugaro cells as the source of serotinin increases frequency IPSPs in Golgi cells ending on the Purkinje cells.

It is very possible that during the registration of long term depression [9] this unique and characteristic type of synaptic plasticity in the cerebellum can play a rather definite role in the rise of its mechanism. So, the Lugaro cells which have been often compared with Golgi cells, ought to be distnguished in a special group of interneurons which contact with Purkinje cells directly and through the action of Golgi cells form their functional significance.

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Կատվի ուղեղիկի կեղեւի Լուգարոյի միջանկյալ բջիջները

Ա. Բ. Մելիք-Մուսյան, Վ. Բ. Ֆանարջյան

Կաթնասունների ուղեղիկի եռաշերտ կեղևը պարունակում է ոչ թե 4 կամ 5 տեսակի բջիջներ, ինչպես նշում էին սրանից 30 տարի առաջ, այլ տարբեր մորֆոլոգիական բնութագրերով և ֆունկցիոնալ նշանակությամբ 8 ինքնատիպ բջիջներ: Այդ նյարդային բջիջների մեջ հատուկ ուշադրության են արժանի գրականությունից արդեն հայտնի Լուգարոյի միջանկյալ բջիջները: Վերջին ժամանակահատվածում մորֆոլոգների և ֆիզիոլոգների մոտ հետաքրքրություն են առաջացնում այս նյարդային բջիջները, չնայած նրանք նկարագրվել են սրանից 100 տարի առաջ: Մեզ այս նյարդաբջիջները հետաքրքրել են ելնելով նրանց արտասովոր կառուցվածքից և միմյանց հետ ունեցած կապերից, ինչպես դա առկա է կատվի ուղեղիկում:

Ներկա աշխատանքի նպատակն է եղել ուսումնասիրել կատվի ուղեղիկի Լուգարոյի բջիջների կառուցվածքը և նրանց կապը ուղեղիկի կեղևի այլ նյարդային գոյացությունների հետ, ինչպես նաև պարզել նրանց դերն ու մասնակցությունը բարդ գործընթացների ամբողջականության մեջ:

Промежуточные клетки Лугаро в коре мозжечка кошки

А.Б.Мелик-Мусян, В.В.Фанарджян

В трехслойной коре мозжечка млекопитающих содержится не 4 или 5 типов клеток, как считалось 30 лет назад, а 8 самостоятельных типов нейронов с различными морфологическими характеристиками и, следовательно, различного функционального назначения. Среди этих клеток особое внимание привлекают

известные в литературе интермедиатные (промежуточные) клетки Лугаро. В последнее время значительный интерес морфологов и физиологов вызывают именно эти нейроны, хотя они были описаны сто лет назад. Нас также заинтересовали эти нейроны в силу их структурной необычности и контактов, которые наблюдаются в мозжечке кошки. Целью данной работы явилось изучение структуры клеток Лугаро, их связей с другими нервными элементами коры мозжечка и их роли в участии сложных процессов интеграции.

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