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A candidate projective neuron type of the cerebellar cortex: the synarmotic neuron

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Dedicated to the outstanding Anatomist and Neuroscientist Professor Glauco Lucio Ambrosi, in memoriam.

ABSTRACT

Previous studies on the granular layer of the cerebellar cortex have revealed a wide distribution of different subpopulations of less known large neuron types, called "non-traditional large neurons", which are distributed in three different zones of the granular layer. These neuron types are mainly involved in the formation of intrinsic circuits inside the cerebellar cortex. A subpopulation of these neuron types is represented by the synarmotic neuron, which could play a projective role within the cerebellar circuitry. The synarmotic neuron cell body map within the internal zone of the granular layer or in the subjacent white substance. Furthermore, the axon crosses the granular layer and runs in the subcortical white substance, to reenter in an adjacent granular layer, associating two cortico-cerebellar regions of the same folium or of different folia, or could projects to the intrinsic cerebellar nuclei. Therefore, along with the Purkinje neuron, the traditional projective neuron type of the cerebellar cortex, the synarmotic neuron is candidate to represent the second projective neuron type of the cerebellar cortex. Studies of chemical neuroanatomy evidenced a predominant inhibitory GABAergic nature of the synarmotic neuron, suggesting that it may mediate an inhibitory GABAergic output of cerebellar cortex within cortico-cortical interconnections or in projections towards intrinsic cerebellar nuclei. On this basis, the present minireview mainly focuses on the morphofunctional and neurochemical data of the synarmotic neuron, and explores its potential involvement in some forms of cerebellar ataxias.

Key words: cerebellum; granular layer; projective neurons; non-traditional large neurons; synarmotic neuron; GABA; monoamines; neuropeptides.

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Introduction

Five neuron types are commonly involved in cerebellar cortex microcircuitry, which include four associative interneurons and one projective neuron type.[1-6] These traditional neuron types include the inhibitory GABAergic stellate and basket neurons which map within the molecular layer,[7-9] the excitatory glutamatergic granule neuron, widely distributed within the granular layer,[10,11] the inhibitory GABAergic Golgi neuron, located within the external granular layer, and the inhibitory GABAergic projective Purkinje neuron, localized in the homonymous layer.[7-9] In addition, in a number of studies focusing on the cerebellar cortex granular layer, different neuron types were identified, apart from the granules and the Golgi neuron.[12-31] These cerebellar neuron types have been referred to as "non-traditional large neurons", since they are not usually considered in the morphofunctional analysis of the cytoarchitectural structure of the cerebellar cortex, and volume of their cell bodies displayed a main diameter of 20-30 µm, in comparison with the one of granules cell bodies which only measures 5-8 µm.[16,17,21-23] Moreover, an excitatory glutamatergic neuron type should also be included among them, the unipolar brush neuron, also called monodendritic neuron, present only in the granular layer of the vestibulocerebellum, which plays a role in the intrinsic cerebellar cortex excitatory circuits.[13,19,27-29] Furthermore, at least nine inhibitory GABAergic large neuron types are distributed in three different zones of the layer and ubiquitously in the various cerebellar lobules, whose role in the cerebellar cortex circuitry is not completely known.[16-18, 21-23,30] In addition, among these large neuron types only the Lugaro neuron, the candelabrum neuron, the globular neuron and the perivascular neuron are involved in specific intrinsic GABAergic inhibitory circuitries of the cerebellar cortex.[16-18,21-23,31] Against this backdrop, the synarmotic neuron must be considered differently, as it is the only inhibitory GABAergic non-traditional large neuron type to which a role in extrinsic (projective) cerebellar circuits is attributed.[16,17,21-23,32-36]

In this minireview we analyze in detail the morphofunctional and neurochemical data on the synarmotic neuron, so far reported. Moreover, in this context, we evaluate the possible involvement of such neuron in an immune-mediated form of cerebellar ataxia.

Synarmotic neuron morphological data

Pioneering morphological studies on cerebellar cortex mentioned the presence, within the granular layer, of large neuron types among the traditional neuronal constituents, characterized by a long axon and extending in the subcortical white substance.[37-47]

Passing through Nissl and Golgi staining studies, Landau[41] first revealed the presence of a specific large neuron type, called synarmotic neuron, in the internal zone of the granular layer or in the subjacent white substance of the cerebellar cortex of several mammals including humans (Figures 1-3).[42-45] Morphological Golgi staining studies confirmed the presence of synarmotic neuron in the internal zone of the granular layer as well as in the subcortical white substance.[46,47] Since the first studies on the synarmotic neuron, it was tought to be implicated in cortico-cortical associative and in cortico-nuclear projective circuits, and recent studies confirmed the projective role of the synarmotic neurons.[9,16,17,21-23,32-36,46,48,49]

Even though the existence of a second projective neuron type in the cerebellar cortex attracted the interest of several neuroscientists[1,4,38-41,50] in most cases this aspect was neglected or not fully considered. Moreover, although in the last twenty-five years several morphological studies through different staining methods supported the presence of a second putative projective large neuron type in the internal zone of the cerebellar granular layer of the mammals including humans, the synarmotic neuron of Landau was not deeply mentioned.

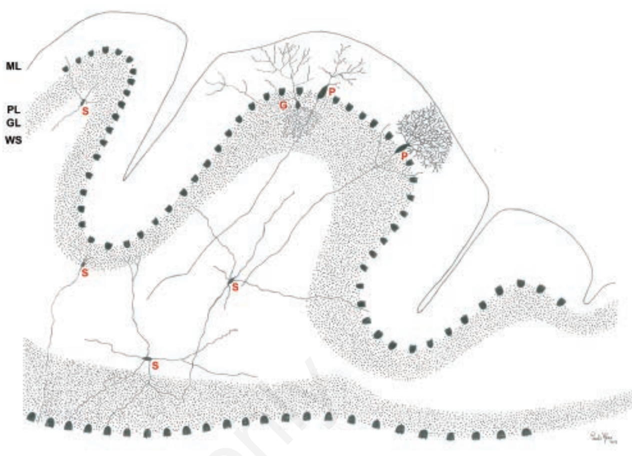

Figure 1. The Anatomic Table by Eber Landau[44] on the synarmotic neuron in the human cerebellar cortex (modified by Paolo Flace, 2021). Table of transverse section of human cerebellar cortex according to the information provided by the Golgi and by Ramon y Cajal silver staining. Morphological structure of the cerebellar cortex: ML, molecular layer; PL, Purkinje neuron layer; GL, granular layer; WS, white substance. Cortico-cerebellar neurons present in the modified anatomic table: P, Purkinje neuron; G, Golgi neuron; S, synarmotic neuron. The cell bodies of different synarmotic neurons are distributed in the internal zone of the granular layer and in the subcortical white substance.

Figure 2. The Anatomic Table by Eber Landau[44] on the synarmotic neuron in the human cerebellar cortex (modified by Paolo Flace, 2021). Table of transverse section of human cerebellar cortex according to the information provided by the Golgi silver staining. Morphological structure of the cerebellar cortex: ML, molecular layer; PL, Purkinje neuron layer; GL, granular layer; WS, white substance. Cortico-cerebellar neurons present in the modified anatomic table: P, Purkinje neuron; G, Golgi neuron; S, synarmotic neuron. A synarmotic neuron cell body localized in the subcortical white substance interposed between two adjacent granular layers, its processes are distributed in both the granular layers.

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Through a modified Golgi staining method, Braak and Braak[12] detected a large neuron type in the internal zone of the granular layer of the human cerebellar cortex, which displayed similar morphological features compared to the synarmotic neuron, and they included it in the type 2 of the classification.

In a study made by using the modified Ehrlich's methylene blue supravital staining technique, the presence of a large neuron type with a stellate-shaped cell body and with a long axon descending into the subcortical white substance was observed in the granular layer of the mouse cerebellar cortex.[47]

The synarmotic neuron exhibits a pear or ovoidal-shaped cell body, with a main diameter ranging from 20 to 25 µm, localized in the internal zone of the granular layer or just below the subcortical white substance (Figures 1-3; Table 1). Its dendritic tree arises from various sites of the cell body and it mainly develops in the internal zone of the granular layer, although, in some cases, the dendrites can run across and radiate within the white substance or direct their extensions into the two granular layers on both sides of the folium white substance (Figures 1-3; Table 1). The axon of the synarmotic neurons originates through the axon hillock from the cell body and runs for a long distance through the white substance, intermingled with efferent and afferent axons to the cerebellar cortex. Subsequently, the axon undertakes different types of growth: it can reenter in the cerebellar cortex so as to associate two adjacent cortico-cerebellar regions of the same folium or different folia; alternatively it may play a role in projective circuits towards the intrinsic cerebellar nuclei (Figures 1-3; Table 1). In relation to the synarmotic neuron afferents, few data are available: it may receive excitatory glutamatergic inputs from mossy fibers (Figures 1-3; Table 1),[2,16,17,21-23,42-45,48] hence the synarmotic neuron dendrites may contribute to the formation of the cerebellar glomeruli, the synaptic complex of the granular layer.[2,16,17,21-23,50,51] It is also likely that its dendrites may receive inhibitory GABAergic afferents from axonal collaterals from other non-traditional large neuron types (*e.g.*, globular neuron; Table 1).[16,17,21-23,52,53] Although, in studies on the cerebellar cortex of several mammals including humans, the presence of the synarmotic neuron has been demonstrated,[9,12,16,17,21-23,32,33,35,36,42-49,52,54] at present time, only few studies have evaluated the quantitative data of the synarmotic neuron. In a quantitative analysis it has been suggested that the synarmotic neuron repre-

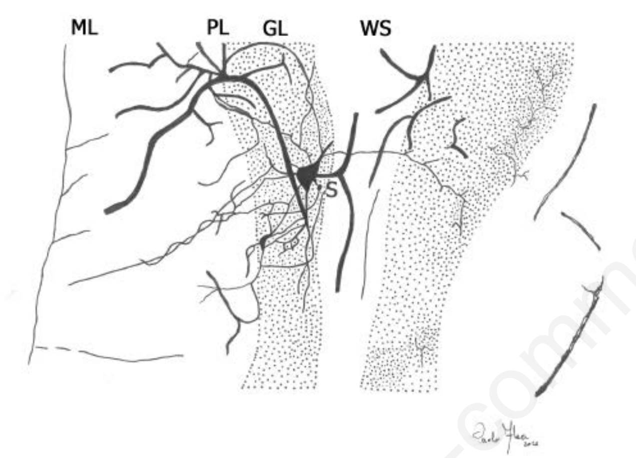

Figure 3. The Anatomic Table by Eber Landau[42] on the synarmotic neuron in the dog cerebellar cortex (modified by Paolo Flace, 2021). Table of transverse section of dog cerebellar cortex according to the information provided by the Golgi and by Ramon y Cajal silver staining. Morphological structure of the cerebellar cortex: ML, molecular layer; PL, Purkinje neuron layer; GL, granular layer; WS, white substance. Cortico-cerebellar neurons present in the modified anatomic table: S, synarmotic neuron. A synarmotic neuron cell body localized in the internal zone of the granular layer, its processes are distributed in two adjacent granular layers.

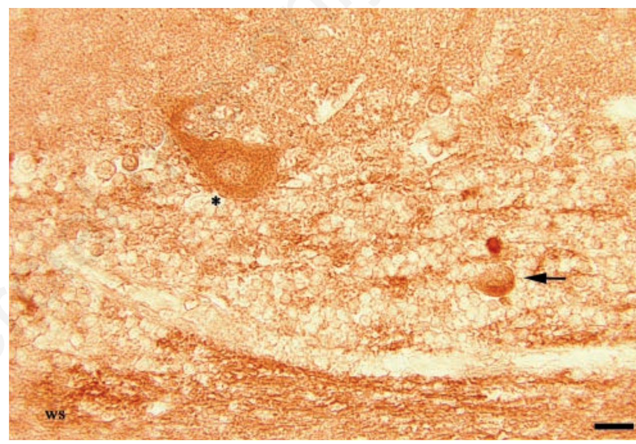

Figure 4. GAD$_{65/67}$ immunoreactive synarmotic neurons in the granular layer of the human cerebellar cortex. GAD$_{65/67}$ immunoreactive synarmotic neuron cell body (arrow) localized in the granular layer in close proximity to the subcortical white substance (WS), from the cell body originates a thin axon-like process. GAD$_{65/67}$ immunoreactive Purkinje neuron cell body and primary dendritic trunk (asterisk); magnification 40x; scale bar: 15 µm. Method: streptavidin-biotin-peroxidase immunoenzymatic technique; chromogen: 3-amino-9-ethyl-carbazole; type of antibody: GAD$_{65/67}$ rabbit polyclonal antibody; Chemicon International, Temecula, CA, USA. Negative control: the sections are covered with normal rabbit serum or with anti-GAD antiserum absorbed with an excess of synthetic GAD (Chemicon International) in place of the primary antibody (original unpublished micrographs; for protocol details, see[16]).

Table 1. Morphological parameters of the synarmotic neuron.

Cell body localization	Features of cell body	Features of the processes	Role in the cerebellar circuitry	References
a) In all cerebellar lobules	a) Ovoidal or pear-shaped b) Horizontal oriented c) Diameter of 20-25 µm	a) The dendrites spread in the granular layer and/or in the white substance	a) Cortico-cortical interconnections	Landau[42-44] Müller[47] Flace *et al.*[16]
b) In the internal zone of the granular layer or in the subcortical white substance		b) The axon runs at the limit of the granular layer or within the white substance	b) Projections towards intrinsic cerebellar nuclei	Ambrosi *et al.*[17] Flace[21-23]

sents a rare variant,[52] while, in another quantitative evaluation, the synarmotic neuron to equal half of the number of Golgi neurons was found.[2] In a study of chemical neuroanatomy on the inhibitory GABAergic non-traditional large neuron types in the granular layer of the human cerebellar cortex by using an antibody raised against the glutamic acid decarboxylase isoforms 65/67 ($GAD_{65/67}$), the GABA biosynthetic enzymes, it was evaluated that the 19.8% of the $GAD_{65/67}$ immunoreactive large neurons localized in the internal zone of the granular layer or in the subcortical white substance corresponds to the synarmotic neurons.[16]

Neurochemical data on the synarmotic neuron

Only few morphofunctional data on the neurotransmitters and neuromodulators expressed by synarmotic neurons are available. In chemical neuroanatomy studies mainly performed on the human cerebellar cortex, the inhibitory GABAergic nature of a subpopulation of synarmotic neuron localized in the internal granular layer (Figure 4) or in the subcortical white matter has been elucidated (Figure 4; Table 2).[16,17,21-23]

Still in developing as well as in the adult human cerebellar cortex, a subpopulation of calbindin D-28k, immunoreactive synarmotic neurons has been detected (Table 2).[48,51] Moreover, in the cerebellar cortex of dromedary camel, calretinin immunoreactive subpopulation of synarmotic neurons has been observed (Table 2).[54] In addition, the presence of different neuropeptidergic subpopulations of synarmotic neurons immunoreactive for vasoactive intestinal polypeptide and motilin (Table 2),[23,55,56] neurotensin and the neurotensin receptor type 1 (NTR_1) has been demonstrated (Table 2).[21-23,34,35] Moreover, recently, the monoaminergic nature of the synarmotic neuron has been also demonstrated. In several studies the presence of two distinct subpopulations of monoaminergic synarmotic neurons has been detected, respectively indolaminergic synarmotic neurons, serotonin (5-HT) immunoreactive (Figure 5; Table 2).[21-23,34,35] and dopaminergic synarmotic neurons, dopamine

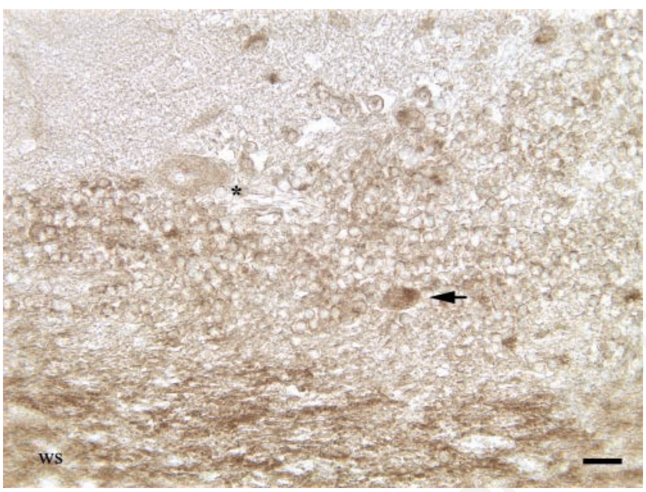

Figure 5. 5-HT immunoreactive synarmotic neuron in the granular layer of the human cerebellar cortex. 5-HT immunoreactive synarmotic neuron cell body localized in the internal zone of the granular layer (arrow) among immunonegative granules, in proximity to the subcortical white substance (WS); magnification 40x; scale bar: 20 µm. Method: streptavidin-biotin-peroxidase immunoenzymatic technique; chromogen: 3,3′-Diaminobenzidine; type of antibody: 5-HT rabbit polyclonal antobody, Covance Lab., Inc., Princeton, NJ, USA. Positive control: fragments of rat duodenum and brainstem are subjected to the same experimental procedures (original unpublished micrographs; for protocol details, see[23]).

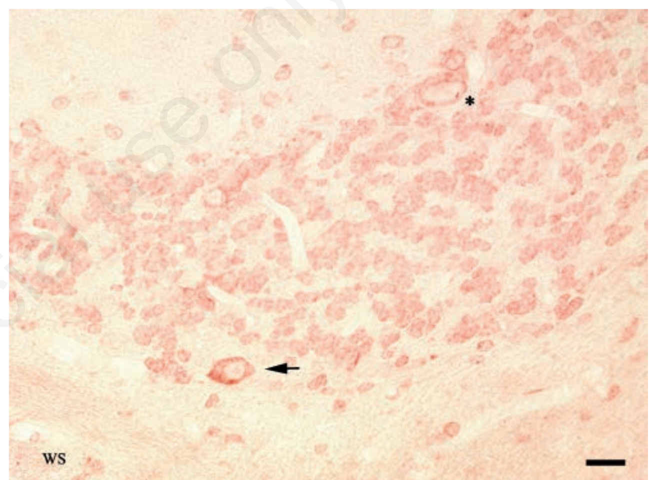

Figure 6. Contactin-1 immunoreactive synarmotic neuron in the mouse cerebellar cortex. Contactin-1 immunoreactive synarmotic neuron cell body (arrow) localized in the internal zone of the granular layer, in close proximity to the subcortical white substance (WS). Contactin-1 immunoreactive Purkinje neuron cell body (asterisk); magnification 40x; scale bar: 20 µm. Method: streptavidin-biotin-peroxidase immunoenzymatic technique; chromogen: 3-amino-9-ethyl-carbazole; type of antibody: Contactin-1 rabbit polyclonal antibody (original unpublished micrographs; for protocol details, see[59]).

Table 2. Neurochemical data of the synarmotic neuron.

Neurochemical parameters	Intensity staining of the cell body	Intensity staining of the dendrites	Intensity staining of the axon	References
Glutamic acid decarboxylase 65/67 ($GAD_{65/67}$)	+++	+++	+++	Flace *et al.*[16]
Vasoactive intestinal polypeptide (VIP)	+++	++	++	Benagiano *et al.*[56]
Calbindin D-28k	+++	+++	+++	Flace *et al.*[48]
Serotonin (5-HT)	+++	+ +	++	Flace[22]
Dopamine transporter (DAT)	+++	+++	+++	Flace *et al.*[58]
Neurotensin	+++	+ +	++	Flace[23]
Contactin-1	+++	++	+	Flace *et al.*[34]

+++, intense; ++, medium; +, weak.

transporter (DAT) immunoreactive (Table 2).[21-23,32-35,56-58] Ultimately, in the adult mouse cerebellar cortex, a subpopulation of synarmotic neurons is immunoreactive for Contactin-1, a cell adhesion glycoprotein involved during the cerebellar development in cellular proliferation and differentiation mechanisms, while, in the adult it plays a role in the regulation of trans-synaptic mechanisms (Figure 6; Table 2).[34,35,59-61]

Potential significance of synarmotic neuron in immune-mediated cerebellar ataxias

Recently, an immune-mediated cerebellar ataxias characterized by the presence of autoantibodies (Auto-Abs) against the GABA biosynthetic enzymatic isoform, glutamic acid decarboxylase isoform 65 (GAD_{65}-Auto-Abs),[62-67] has been described. In this form of cerebellar ataxia, the GAD_{65}-Auto-Abs[62,63,65,68-74] cause a damage of the cerebellar cytoarchitecture and a dysregulation of the inhibitory signaling of the traditional GABAergic corticocerebellar neurons (*e.g.*, stellate neurons, basket neurons, Purkinje neurons) involved in associative and in projective cerebellar inhibitory circuits.[63,64,66,70-72]

In this regard, a dysregulation of the inhibitory GABAergic synarmotic neurons circuitries (Figure 7),[16,17,21-23] could be involved in the impairment of the inhibitory GABAergic signaling implied in this GAD_{65}-Auto-Abs mediated form of cerebellar ataxia[62,63,68,71,73] and could play a foundamental role in the symptomatic manifestations of this subtype of cerebellar ataxia.[62,63,68-73]

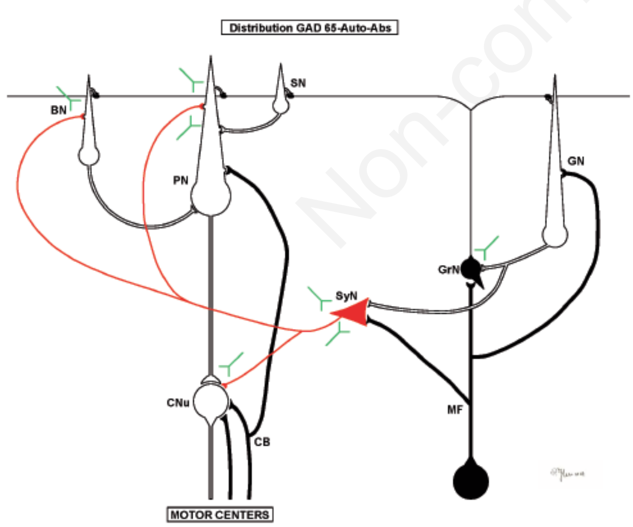

Figure 7. GAD_{65}-auto-antibodies in the cerebellum. Scheme on the distribution of the autoantibodies against the GABA biosynthetic enzymatic isoform, glutamic acid decarboxylase isoform 65 (GAD_{65}-Auto-Abs) related to the GABAergic synaptic sites of the synarmotic neuron in the cerebellum. BN, basket neuron; PN, Purkinje neuron, SN, stellate neuron; GN, Golgi neuron; GrN, granule; Syn, synarmotic neuron; CNu, cerebellar nuclei; CB, climbing fibers; MF, mossy fibers.

Concluding remarks

This minireview supplies, for the first time, several morpho-functional aspects on the assumptive projective neuron type of the cerebellar cortex, the synarmotic neuron also called the Landau neuron,[41-46] one of the neglected inhibitory GABAergic non-traditional large neuron types of the cerebellar cortex.[16,17,21-23] In addition, the neurochemical data of the synarmotic neuron and its possible involvement in the impairment of GABAergic neurotransmission mechanisms of the anti-GAD_{65}-Auto-Abs immune-mediated cerebellar ataxia has been also analized.

Conclusively, in this minireview the presence of the subpopulation of synarmotic neurons in the mammal cerebellar cortex (including humans), has been established, and various neurochemical aspects have been also analyzed. However, yet further detailed research is needed for understanding data on the development of the synarmotic neuron, and on its functional role in neurotransmission mechanisms and in cerebellar disorders.

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References

1. Jansen J, Brodal A. [Das Kleinhirn] In: Bargman W, editor. [Handbuch der Mikroskopiscen Anatomie des Menschen].[Book in German]. Berlin, Springer; 1958. pp 91-149.
2. Eccles JC, Ito M, Szentàgothai J. The cerebellum as a new neuronal machine. Springer, Berlin; 1967.
3. Fox CA, Snider RS, The cerebellum. Amsterdam, Elsevier; 1967.
4. Mugnaini, E. The histology and cytology of the cerebellar cortex. In: Larsell O, Jansen J. editors. The comparative anatomy and histology of the cerebellum: the human cerebellum, cerebellar connections and cerebellar cortex. Minneapolis, Minnesota Press; 1972. pp 201-64.
5. Ito M. The cerebellum and neural control. New York, Raven Press; 1984.
6. Ito M. The cerebellum: brain for an implicit self. Upper Saddle River, FT Press: 2011.
7. Gabbott PL, Somogyi P, Stewart MG, Hamori J. GABA-immunoreactive neurons in the rat cerebellum: a light and electron microscope study. J Comp Neurol 1986;251:474-90.
8. Flace P, Benagiano V, Virgintino D, Rizzi A. [L'immunocitochimica per GAD e GABA rivela elementi GABAergici della corteccia cerebellare di uomo].[in Italian] Proc. 7th Nat. Congr. Young Investigator of Neuroscience, Pisa; 2000; p. 10.
9. Benagiano V, Flace P, Virgintino D, Rizzi A, Roncali L, Ambrosi G. Immunolocalization of glutamic acid decarboxylase in postmortem human cerebellar cortex. A light microscopy study. Histochem Cell Biol 2000;114:191-5.
10. Clements JR, Monaghan PL, Beitz AJ. An ultrastructural description of glutamate-like immunoreactivity in the rat cerebellar cortex. Brain Res 1987;421:343-8.
11. Ottersen OP, Laake JH, Storm-Mathisen J. Demonstration of releasable pool of glutamate in cerebellar mossy and parallel

fibre terminals by means of light and electron microscopic immunohistochemistry. Arch Ital Biol 1990;128:111-25.
12. Braak E, Braak H. On three types of large nerve cells in the granular layer of the human cerebellar cortex. Anat Embryol (Berl) 1983;166:67-86.
13. Mugnaini E, Floris A. Unipolar brush cell: a neglected neuron of the mammalian cerebellar cortex. J Comp Neurol 1994;339:174-80.
14. Geurts FJ, Timmermans J, Shigemoto R, De Schutter E. Morphological and neurochemical differentiation of large granular layer interneurons in the adult rat cerebellum. Neuroscience 2001;104;499-512.
15. Geurts FJ, De Schutter E, Dieudonné S. Unraveling the cerebellar cortex: cytology and cellular physiology of large-sized interneurons in the granular layer. Cerebellum 2003;2:290-9.
16. Flace P, Benagiano V, Lorusso L, Girolamo F, Rizzi A, Virgintino D, et al. Glutamic acid decarboxylase immunoreactive large neuron types in the granular layer of the human cerebellar cortex. Anat Embryol (Berl) 2004;208:55-64.
17. Ambrosi G, Flace P, Lorusso L, Girolamo F, Rizzi A, Bosco L, et al. Non-traditional large neuron in the granular layer of the cerebellar cortex. Eur J Histochem 2007;51:59-64.
18. Schilling K, Oberdick J, Rossi F, Baader SL. Besides Purkinje cells and granule neurons: an appraisal of the cell biology of the interneurons of the cerebellar cortex. Histochem Cell Biol 2008;130:601-15.
19. Mugnaini E, Sekerkova G, Martina M. The unipolar brush cell a remarkable neuron finally receiving deserved attention. Brain Res Rev 2011;66:220-45.
20. Jacobs B, Johnson NL, Wahl D, Schall M, Maseko BC, Lewandowski A, et al. Comparative neuronal morphology of the cerebellar cortex in afrotherians, carnivores, cetartiodactyls and primates. Front Neuroanat 2014;8:24.
21. Flace P. The neglected non-traditional large neuron types in the granular layer of the cerebellar cortex: Morphofunctional and neurochemical data. Ital J Anat Embryol 2017;122:225.
22. Flace P. New immunohistochemical data on the non-traditional large neuron types of the granular layer of the human cerebellar cortex. Proc. 65th Congr. Italian Embryological Group (GEI - SIBSC) - 38th Congr. Italian Society of Histochemistry (SII), Ancona, 2019; p. 14. Eur J Histochem 2019;63:3052.
23. Flace P. Neurochemical data on the non-traditional large neuron types of the granular layer of the human cerebellar cortex. Proc. 29th Nat Conf Italian Group for the Study of Neuromorphology (GISN), p. 2. Eur J Histochem 2020;64: 3121.
24. Flace P, Milardi D, Basile GA, Gennarini G, Anastasi G. A light microscopy study on the neurotensiergic system in the human cerebellum. Proc. 28th Nat. Congr. Italian Group for the Study of Neuromorphology (GISN), Florence, 2018; p.16.
25. Flace P, Anastasi G, Milardi D, Basile GA, Quartarone A, Cacciola A. The human cerebellar dopaminergic system, its links to the midbrain dopaminergic nuclei and role in neurologic and psychiatric diseases. Neurol Sci 2019;40:S262.
26. Mugnaini E. GABAergic inhibition in the cerebellar system. In: Martin DL, Olsen RW, editors. GABA in the nervous system: the view at fifty years. Philadelphia, Lippincott; 2000. pp 383-407.
27. Altmann J, Bayer SA. Time of origin and distribution of a new cell type in rat cerebellar cortex. Exp Brain Res. 1977;29:265-74.
28. Munoz DG. Monodendritic neurons: a cell type in the human cerebellar cortex identified by chromogranin A-like immunoreactivity. Brain Res 1990;528:335-8.
29. Braak E, Braak H. The new monodendritic neuronal type within the adult human cerebellar granule cell layer shows calretinin-immunoreactivity. Neurosci Lett 1993;154:188-202.
30. Crook J, Hendrickson A, Robinson, FR. Co-localization of glycine and gaba immunoreactivity in interneurons in Macaca monkey cerebellar cortex. Neuroscience 2006;141:1951-9.
31. Hirono M, Nagao S, Yanagawa Y, Konishi S. Monoaminergic modulation of GABAergic transmission onto cerebellar globular cells. Neuropharmacology 2017;118:79-89.
32. Flace P, Milardi D, Cacciola A, Basile G. Multidisciplinary morphological approaches to the intrinsic human cerebellar dopaminergic system, its projection and clinical role. Ital J Anat Embryol 2018;123:91.
33. Flace P, Cacciola A, Milardi D, Basile GA, Anastasi G. Immunohistochemical and tractographic approaches on the human cerebellar dopaminergic system. Proc. 65th Congr. Italian Embryological Group (GEI - SIBSC) - 38th Congr. Italian Society of Histochemistry (SII), Ancona, 2019; p. 14. Eur J Histochem 2019;63:3052.
34. Flace P, Bizzoca A, Livrea P, Gennarini G. The synarmotic neuron. A candidate projective neuron type of the cerebellar cortex. Neurol Sci 2022;43:S432.
35. Flace P, Galletta D, Bizzoca A, Gennarini G, Livrea P. New immunohistochemical data on the cerebellar synarmotic neuron type. Proc. 28th Nat. Congr. Italian Group for the Study of Neuromorphology (GISN), Florence, 2018; p.16.
36. Golgi C. Sulla fina anatomia del Sistema nervoso. IV. Sulla fina anatomia delle circonvoluzioni cerebellari. Rivista Sperimentale di Freniatria e Medicina Legale delle Alienazioni Mentali 1883;9:1-17.
37. Retzius G. [Kleinere Mitteeilungen von dem Gebiete der Nervenhistologie].In: [I. Ueber die Golgi'schen Zellen und die Kletterfarsen Ramón y Cajal's in der Kleinhirnrinde. Biologische Untersuchungen].[Book in German]. Stockholm, Neue Folge, Samson & Wallin; 1892. p 57-9.
38. Kölliker A. [Handbuch der Gewebelehre des Menschen. Nervensystem des Menschen und der Tiere].[Book in German]. Leipzig, Wilhelm Engelmann; 1896.
39. Jakob A. [Das Kleinhirn]. In: von Möllendorff W, editor. [Handbuch der Mikroskopischen Anatomie des Menschen].[Book in German]. Cham, Springer; 1928. p 674-916.
40. Ramón y Cajal S. [Histologie du systéme nerveux de l'homme et des vertébrés].[Book in French]. Paris, Maloine; 1911.
41. Landau E. [Beitrag zur Kenntnis der Körnerschicht des Kleinhirns].[Article in German]. Vorl Mitt Anat Anz 1927;62:391-8.
42. Landau E. [Über cytoarchitektonische Bauunterschiede in der Körnerschicht des Kleinhirns].[Article in German]. Z Anat 1928;87:551-7.
43. Landau E. [La cellule synarmotique].[Article in French]. Bull Histol Appl 1932;9:159-68.
44. Landau E. [La cellule synarmotique dans le cervelet humain].[Article in French]. Arch Anat 1933;17:273-85.
45. Kesiunaité D. [Quelques mots sur la structure de la couche granuleuse du cervelet].[Article in French]. Bull Histol Appl 1930;7:156-61.
46. Löwenberg H. The presence of the synarmotical cell in the cerebellum of birds. Bio Morph 1938;1:273-80.
47. Müller T. Large nerve cells with long axons in the granular layer and white matter of the murine cerebellum. J Anat 1994;184:419-23.
48. Flace P, Lorusso L, Laiso G, Rizzi A, Cagiano R, Nico B, et al. Calbindin-D28k immunoreactivity in the human cerebellar cortex. Anat Rec 2014;297:1306-15.
49. Chan-Palay V. Fine structure of labelled axons in the cerebellar

cortex and nuclei of rodents and primates after intraventricular infusions with tritiated serotonin. Anat Embryol (Berl) 1975;148:235-65.
50. Hámori J, Mezey E. Serial and triadic synapses in the cerebellar nuclei of the cat. Exp Brain Res 1977;30:259-73.
51. Katsetos CD, Frankfurter A, Christakos S, Mancall EL, Vlachos IN, Urich H. Differential localization of class III, betatubulin isotype and calbindin-D28k defines distinct neuronal types in the developing human cerebellar cortex. J Neuropathol Exp Neurol 1993;52:655-66.
52. Lainé J, Axelrad H. Extending the cerebellar Lugaro cell class. Neuroscience 2002;115:363-74.
53. Flace P, Cacciola A, Milardi D, Basile GA, Anastasi G. Immunohistochemical and tractographic approaches on the human cerebellar dopaminergic system. Proc. 28th Nat. Congr. Italian Group for the Study of Neuromorphology (GISN), Florence, 2018; p. 14.
54. Attaai AH, Noreldin AE, Abdel-maksoud FM, Hussein MT. An updated investigation on the dromedary camel cerebellum (Camelus dromedarius) with special insight into the distribution of calcium-binding proteins. Sci Rep 2020;10:21157.
55. Flace P, Lorusso L, Benagiano V, Ambrosi G. Double labelling immunocytochemical tecniques reveal colocalizations of GAD with motilin or VIP in the human cerebellar cortex. Proc. 15th Na.t Congr. Italian Group for the Study of Neuromorphology (GISN), Bologna, 2005; p. 18. Acad Sci Bologna Inst 2006.
56. Benagiano V, Flace P, Lorusso L, Rizzi A, Bosco L, Cagiano R, Ambrosi G. Vasoactive intestinal polypeptide immunoreactivity in the human cerebellum: qualitative and quantitative analyses. J Anat 2009;215:256-66.
57. Flace P, Livrea P, Galletta D, Gulisano M, Gennarini G. Translational study of the human cerebellar dopaminergic system, its interconnections and role in neurologic and psychiatric disorders. Proc. 30th Nat. Conf. Italian Group for the Study of Neuromorphology (GISN), Torino, 2020; p. 15. Eur J Histochem 2020;64:3200.
58. Flace P, Livrea P, Basile G, Galletta D, Bizzoca A, Gennarini G, et al. The cerebellar dopaminergic system. Front Syst Neurosci 2021;15:650614.
59. Bizzoca A, Virgintino D, Lorusso L, Buttiglione M, Yoshida L, Polizzi A, et al. Transgenic mice expressing F3/contactin from the TAG-1 promoter exhibit developmentally regulated changes in the differentiation of cerebellar neurons. Development 2003;130:29-43.
60. Bizzoca A, Corsi P, Gennarini G. The mouse F3/contactin glycoprotein: structural features, functional properties and developmental significance of its regulated expression. Cell Adh Migr 2009;3:53-63.
61. Bizzoca A, Jirillo E, Flace P, Gennarini G. Overall role of contactins expression in neurodevelopmental events and contribution to neurological disorders. CNS Neurol Disord Drug Targets 2022;22:1176-93.
62. Honnorat J, Saiz A, Giometto B, Vincent A, Brieva L, de Andres C, et al. Cerebellar ataxia with anti-glutamic acid decarboxylase antibodies: study of 14 patients. Arch Neurol 2001;58:225-30.
63. Manto M, Honnorat J, Hampe CS, Guerra-Narbona R, López-Ramos JC, Delgado-Garcia JM, et al. Disease-specific monoclonal antibodies targeting glutamate decarboxylase impair GABAergic neurotransmission and affect motor learning and behavioral functions. Front Behav Neurosci 2015;9:78.
64. Manto M. Mitoma H, Hampe CS. Anti-GAD Antibodies and the cerebellum: where do we stand? Cerebellum 2019;18:153-6.
65. Mitoma H, Hadjivassiliou M, Honnorat J. Guidelines for treatment of immune-mediated cerebellar ataxias. Cerebellum Ataxias 2015;2:14.
66. Mitoma H, Adhikari K, Aeschlimann D, Chattopadhyay P, Hadjivassiliou, M, Hampe CS. et al. Consensus paper: neuroimmune mechanisms of cerebellar ataxias. Cerebellum 2016;15:213-32.
67. Mitoma H, Manto M, Hampe CS. Pathogenic roles of glutamic acid decarboxylase 65 autoantibodies in cerebellar ataxias. J Immunol Res 2017;2017:2913297.
68. Mitoma H, Song SY, Ishida K, Yamakuni T, Kobayashi T, Mizusawa, H. Presynaptic impairment of cerebellar inhibitory synapses by an autoantibody to glutamate decarboxylase. J Neurol Sci 2000;175:40-4.
69. Mitoma H, Ishida K, Shizuka-Ikeda M, Mizusawa, H. Dual impairment of GABAA- and GABAB-receptor-mediated synaptic responses by autoantibodies to glutamic acid decarboxylase. J Neurol Sci 2003;208:51-6.
70. Kono S, Miyajima H, Sugimoto M, Suzuki Y, Takahashi Y, Hishida A. Stiff-person syndrome associated with cerebellar ataxia and high glutamic acid decarboxylase antibody titer. Intern Med 2001;40:968-71.
71. Ishida K, Mitoma H, Wada Y, Oka T, Shibahara J, Saito Y, et al. Selective loss of Purkinje cells in a patient with anti-glutamic acid decarboxylase antibody-associated cerebellar ataxia. J Neurol Neurosurg Psychiatry 2007;78:190-2.
72. Ishida K, Mitoma H, Mizusawa H. Reversibility of cerebellar GABAergic synapse impairment induced by anti-glutamic acid decarboxylase autoantibodies. J Neurol Sci 2008;271:186-90.
73. Joubert B, Rostásy K, Honnorat J. Immune-mediated ataxias. Handb Clin Neurol 2018;155:313-32.
74. Tohid H. Anti-glutamic acid decarboxylase antibody positive neurological syndromes. Neurosciences (Riyadh) 2016;21:215-22.

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