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Glutamic acid decarboxylase immunoreactive large neuron types in the granular layer of the human cerebellar cortex

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Abstract ‘Non-traditional’ large neurons of the granular layer of the cerebellar cortex include all its large neuronal types, except the Golgi neuron, which is instead one of the five ‘classic’ types of corticocerebellar neurons. The morphological, chemical and functional characteristics of the ‘non-traditional’ large neurons have not been entirely ascertained. The aim of this study was to ascertain whether morphological evidence can be provided of GABA synthesis within the ‘non-traditional’ large neurons of the human cerebellar cortex by means of immunocytochemistry for glutamic acid decarboxylase (GAD). Fragments of postmortem cerebellar cortex of various lobules from the hemispheres and vermis were studied. Immunoreactions revealed large neurons distributed throughout the granular layer in all lobules examined. They were discriminated by analyzing the morphological features of their bodies and processes and were identified as Golgi neurons and as some ‘non-traditional’ types, such as the candelabrum, Lugaro and synarmotic neurons. In addition, immunoreactive large neurons, with their bodies and processes closely adjacent to microvessels, were observed throughout the layer: these perivascular neurons could represent a new type of ‘non-traditional’ neuron of the cerebellar cortex. This study supplies the first indication that in the human cerebellar cortex some types of ‘non-traditional’ large neurons are GAD-immu-

noreactive, in addition to those neurons already known to be GABAergic (i.e., stellate, basket, Purkinje and Golgi neurons). These morphological data further point out possible functional roles for GABA as a neurotransmitter/neuromodulator in intrinsic, associative and projective circuits of the cerebellar cortex.

Keywords Cerebellum · Non-traditional neurons · Immunocytochemistry · GABA · Microvessel nervous regulation

Introduction

The granular layer of the cerebellar cortex is usually described as composed of two groups of neurons: the granule cells, small neurons densely packed within the layer, and the large neurons, distributed throughout the layer and scattered among the granule cells, often being surrounded and almost hidden by them. Morphological evidence has indicated that the large neurons of the granular layer are of different types, which include the Golgi neuron, one of the five ‘classic’ corticocerebellar neurons (together with stellate, basket, Purkinje and granule neurons), and a number of other neuron types (Golgi 1874; Lugaro 1894; Pensa 1931; Landau 1933; Jansen and Brodal 1958; Eccles et al. 1967; Mugnaini 1972; Palay and Chan-Palay 1974; Braak and Braak 1983), generically indicated as ‘non-traditional’ neurons of the cerebellar cortex (Mugnaini 2000).

The Golgi neuron has a polygonal or ovoid body localized in the external granular layer and gives rise to dendrites that ascend into the molecular layer, where they principally synapse with parallel fibers, or remain in the granular layer, where they participate in the building of the cerebellar glomeruli. The Golgi neuron axon arises from the internal portion of the body, arborizes in the granular layer and enters the cerebellar glomeruli, where it principally synapses with granule cell dendrites. The main functional role of the Golgi neuron is that of modulation of afferences to the cerebellar cortex trans-

To our Master, Professor Rodolfo Amprino, with our great admiration, gratefulness and affection, on the occasion of his ninety-second birthday

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ported by the mossy fibers (Golgi 1874; Ramón y Cajal 1911; Jansen and Brodal 1958; Eccles et al. 1967; Mugnaini 1972; Palay and Chan-Palay 1974; Voogd and Glickstein 1998; Castejon and Castejon 2000). Immunocytochemical studies have indicated that most of the Golgi neurons use GABA as a chemical neurotransmitter (Mugnaini and Oertel 1985; Ottersen et al. 1988; Benagiano et al. 2000).

Despite several observations indicating that a number of large neurons distinct from the Golgi neurons exist in the granular layer, the so called 'non-traditional' neurons, their morphological identification is still incomplete and their functional role largely unknown. A classification of the 'non-traditional' neurons in the granular layer was firstly attempted by a staining technique that revealed intracellular deposits of lipofuscin and made it possible to identify three large neuron types (type I, II and III). Types II and III included, in turn, different subtypes (Braak and Braak 1983). Afterwards, the heterogeneity of the 'non-traditional' neurons was confirmed by immunocytochemical studies that allowed identification of different types according to their neurochemical characteristics, such as the presence of neurotransmitters/neuromodulators (e.g., GABA, glutamate, somatostatin) (Aoki et al. 1986; Lainé and Axelrad 1998; Geurts et al. 2001; Nunzi et al. 2001), calcium-binding proteins (e.g., calretinin, calbindin D_{28k}) (Braak and Braak 1993; Katsetos et al. 1993; Floris et al. 1994; Fortin et al. 1998; Lainé and Axelrad 2002) and chromogranin A (Munoz 1990).

At the present time, four types of 'non-traditional' neurons have been characterized in the granular layer: the Lugaro neuron, the synarmotic neuron, the candelabrum neuron and the unipolar brush neuron.

The Lugaro neuron (intermediate neuron of Lugaro) has a spindle-shaped body lying parallel to the cerebellar surface and localized in the external granular layer, just beneath the Purkinje neuron body layer. Two dendrite trunks originate from the neuron opposite poles, run along the boundary between the Purkinje neuron body and granular layers, and ramify within the granular layer; the axon and its branches mainly projects towards the molecular layer (Lugaro 1894; Fox 1959; Braak 1974; Christ 1985; Lainé and Axelrad 1996; Melik-Musian and Fanardzhian 1998).

The synarmotic neuron has a body localized in the internal granular layer or even in the subjacent white matter. Its dendritic tree is confined to the granular layer, while the axon enters into the white matter, interposing among axons efferent from, or afferent to, the cerebellar cortex (Landau 1933; Jansen and Brodal 1958).

The candelabrum neuron has a pear-shaped body lying orthogonally to the cerebellar surface and localized in close proximity to the Purkinje neuron body layer. From the apex of the body, corresponding to its external pole, this neuron gives rise to both dendrites and axon, which ascend into the molecular layer in a candelabrum-like fashion (Lainé and Axelrad 1994).

In contrast with the above 'non-traditional' neurons, which have been described in the cortex of all the

cerebellum lobes, the unipolar brush neuron has been mainly observed in the flocculonodular lobe. This neuron has a roundish or ovoid body localized throughout the granular layer. From its body region facing towards the cerebellar surface a single dendrite trunk originates and in turn gives out packed small branches that spread in the molecular layer or in the neighboring granular layer. The axon ramifies within the granular layer, its branches penetrating into cerebellar glomeruli, where they originate 'intrinsic' mossy fibers, in addition to the classic, 'extrinsic' mossy fibers (Altman and Bayer 1977; Mugnaini and Floris 1994; Diño et al. 2000; Houk and Mugnaini 2003).

The amino acid γ -aminobutyric acid (GABA) is the chemical neurotransmitter most widely distributed in the mammalian cerebellar cortex. Neurons localized in all layers of the cerebellar cortex (stellate and basket neurons, in the molecular layer; Purkinje neurons, whose bodies lie in the homonymous layer; and Golgi neurons, in the granular layer) have been demonstrated to be immunoreactive to GABA markers (Ribak et al. 1978; Mugnaini and Oertel 1985; Gabbott et al. 1986; Benagiano et al. 2000; Mugnaini 2000). However, most accounts of the GABA neurons in the granular layer consider only the Golgi neuron and do not make any reference to other neuron types. Only occasionally has a GABAergic nature been attributed to the Lugaro neuron (Aoki et al. 1986; Lainé and Axelrad 1998).

The aim of the present immunocytochemical study was to investigate the presence and distribution of GABA synthesizing neuron types among the large neurons of the granular layer of the human cerebellar cortex. The study was carried out by immunocytochemistry for glutamic acid decarboxylase (GAD), the GABA biosynthetic enzyme, using an antibody raised against a synthetic peptide corresponding to the rat C-terminus residues 572 to 585, present in both the human enzyme isoforms, GAD 65 and GAD 67. In a previous study, we demonstrated that this anti-GAD 65/67 antibody is able to label neuron bodies and processes, as well as axon terminals, so also providing information on the morphology of GABA-synthesizing neurons (Benagiano et al. 2000).

Materials and methods

Cerebella of six adult patients aged between 23 and 61 years, with no clinical history of neurological disease and who died of non-neurological causes, were removed at autopsy within 24 to 36 h after death. Five fragments of 30 to 40 mm³, including the whole cerebellar cortex, were taken from each cerebellum: two from the right and left posterior quadrangular lobules, two from the right and left tonsillae, in the hemispheres, one from the pyramis, in the vermis.

The fragments were fixed by immersion for 2 h in Bouin's fluid devoid of acetic acid, dehydrated in an ethanol series and embedded in a semi-synthetic paraffin wax. Paraffin blocks were serially cut into 5- μ m sections orthogonally oriented towards the cerebellar surface. Sections from each fragment were stained with toluidine blue and submitted to histopathological analysis. No neuropathological changes in nervous tissues were observed at routine histopathological examination.

From each fragment, ten sections selected at intervals of 100 μm were submitted to immunocytochemistry for GAD 65/67. The sections were immunolabeled according to the following procedure: blockade of endogenous peroxidase by treatment with 3% hydrogen peroxide solution in water for 10 min at room temperature (RT); rinsing for 3 \times 10 min in phosphate-buffered saline (PBS), pH 7.6; preincubation in non-immune goat serum (Dako LSAB kit, Dako, CA, USA) for 30 min at RT; incubation with the primary antibody (rabbit polyclonal antibody to GAD 65/67, Chemicon, CA, USA), diluted 1:600 in PBS, overnight at 4°C; rinsing for 3 \times 10 min in PBS; incubation with the biotinylated secondary antibody (Dako LSAB kit, Dako) for 30 min at RT; rinsing for 3 \times 10 min in PBS; incubation with the streptavidin-peroxidase complex (Dako LSAB kit, Dako) for 30 min at RT; incubation with the chromogen 3-amino-9-ethyl-carbazole (AEC) (Vector Laboratories, CA, USA) for 20 min at RT.

Negative controls

Total abolition of the immunolabeling was obtained by treating sections with the non-immune serum or with the primary antibody preadsorbed with an excess of the synthetic peptide used as immunogen.

Quantitative analyses

Morphometric analysis was performed using the image analyzer VIDAS 2.5 (Kontron Elektronik, Eching, Germany) connected via a high-resolution (1600 \times 1200 active pixels) CCD/RGB color video camera (TK-1070E, JVC, Japan) to the Axioscop 20 light microscope (Carl Zeiss, Germany). All GAD-immunolabeled sections were acquired at \times 2.5 magnification and then submitted to a semi-automatic sequence of commands (macro) in order to discriminate the granular layer and evaluate its area in mm^2 . The most important steps of the macro were: image grabbing and digitization to a high-resolution color monitor; image processing, by tracing manually the granular layer contour and setting pixels of the image located inside the contour at a predefined gray value; segmentation by gray value threshold modality to discriminate the granular layer from the background; object identification for object-specific parameter measurements; automatic measurement sequence.

The total area of the granular layer, the numbers of GAD-immunoreactive large neurons and their densities (per mm^2) were then calculated in each cerebellar lobule examined. Only neurons having a main body diameter at least triple that of the granule cells were considered. To facilitate description of the distribution of GAD-immunoreactive large neurons within the granular layer, the latter was subdivided into three zones, external, intermediate and internal, each having equal thickness, whatever the whole granular layer thickness (which ranges between 100 and 500 μm from the base to the summit of the folium).

Qualitative analyses

All GAD-immunolabeled sections and neighboring toluidine blue-stained sections were examined under the light microscope in order to analyze morphologically and classify all the large neurons. The different types of GAD-immunoreactive large neurons were described separately in the three zones of the granular layer. The number of each neuron type identified was expressed as the percent value of the total number of immunoreactive large neurons present in all lobules examined.

Results

In the whole area of granular layer examined (183.5 mm^2), a total number of 1557 GAD 65/67-

immunoreactive large neurons was found. They displayed a main diameter ranging from 20 to 30 μm . Of these neurons, 359 were detected in the right tonsilla (density: 10.1 per mm^2), 345 in the left tonsilla (density: 10.9 per mm^2), 331 in the pyramis (density: 9.1 per mm^2), 274 in the right quadrangular lobule (density: 7.1 per mm^2), and 250 in the left quadrangular lobule (density: 5.9 per mm^2).

GAD-immunoreactive large neurons were detected throughout the granular layer of all lobules examined, scattered among strongly positive puncta, mainly corresponding to axon terminals, and immunonegative granule cells (Fig. 1). Immunoreactivity appeared diffused in the perikaryon and extended into dendrite-like trunks. Immunoreactivity was also observed in axon-like processes, recognizable for their varicosities and usually more strongly labeled than perikarya and dendrites.

We indicated as vertical neurons, the large ones having a body with the main diameter orthogonal to the cerebellar surface, and as horizontal neurons, those having a body with the main diameter parallel to the cerebellar surface.

Different large neuron types were distinguished on the basis of analyses of the morphological features of their bodies and processes carried out on both GAD-immunolabeled and toluidine blue-stained sections. They were described separately in the external, intermediate and internal zones.

GAD 65/67-immunoreactive large neurons in the external zone

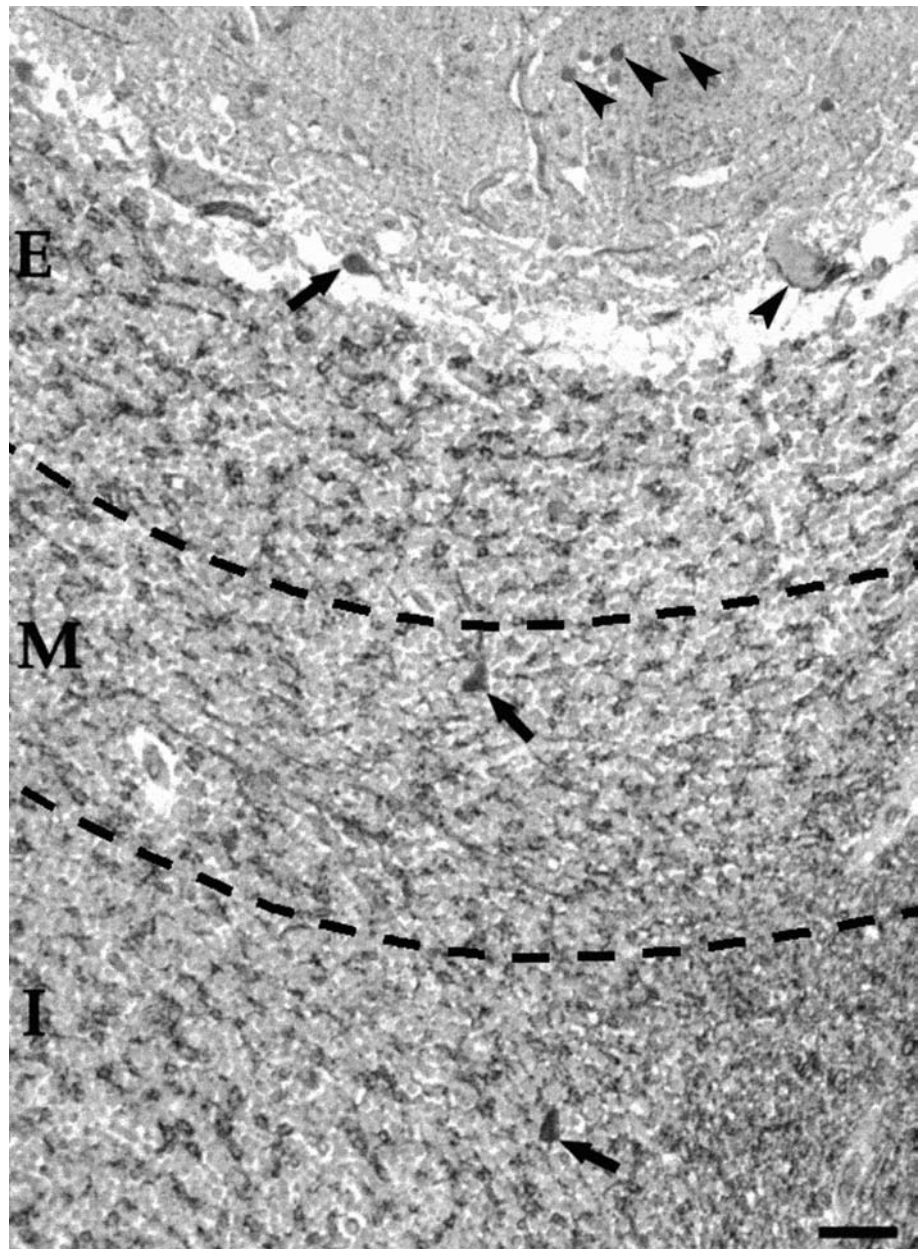
A total of 1304 GAD 65/67-immunoreactive large neurons, corresponding to 83.7% of the positive large neurons, were observed in the external zone of the granular layer. Of these neurons, 40.6% had a vertical, pear-shaped body, whose larger pole was situated internally and contained a nucleus. Their bodies appeared squeezed against immunolabeled Purkinje neuron bodies or intercalated among them. From the smaller, external pole of the body, positive apical processes were directed towards the molecular layer (Fig. 2A, B).

A second neuron type constituted 26.6% of the immunoreactive large neurons. This neuron showed a polygonal or ovoid, vertical body and a positive axon-like process emerging from the internal portion of the body and directed towards the clusters of positive axon terminals throughout the layer (Fig. 2C).

A third type (23.4%) was characterized by a horizontal, spindle-shaped body. Its dendrite-like processes arising from the opposite poles of the body were seen to run horizontally along the boundary between the granular and Purkinje neuron body layers (Fig. 2D).

The remaining 9.4% were of perivascular type, characterized by a roundish body with an extensive surface leaning against the wall of microvessels (Fig. 2E).

Fig. 1 GAD 65/67 immunoreactivity in the granular layer of the human cerebellar cortex. For convenience, two *stippled lines* mark the boundaries among the external (*E*), intermediate (*M*) and internal (*I*) zones. In the granular layer, immunoreactive large neurons (*arrows*) are scattered among immunonegative granule cells and clusters of positive puncta, putative GABAergic axon terminals. Some immunoreactive large neurons (*arrowheads*) are also detectable in the other two layers of the cerebellar cortex. *Scale bar*, 50 μ m



GAD 65/67-immunoreactive large neurons in the intermediate zone

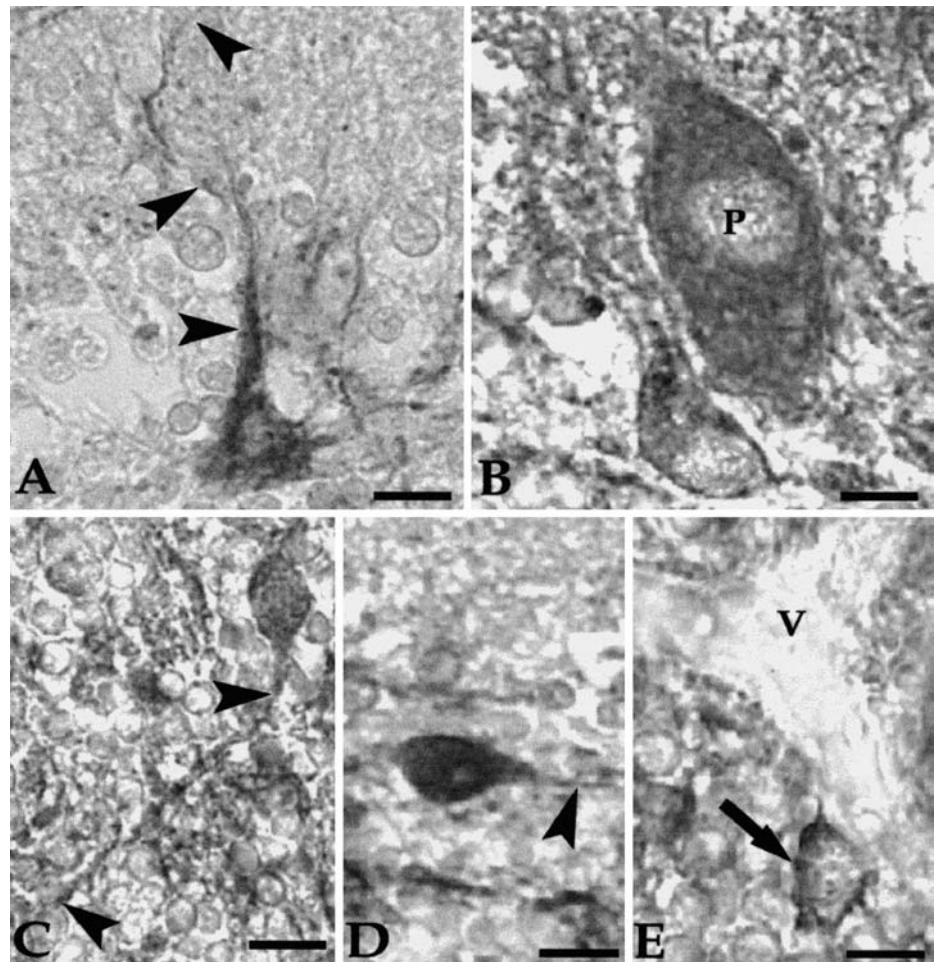
A total of 174 immunoreactive large neurons (11.2%) were found in the mid-granular layer. Of these neurons, 31.5% showed a triangular body with the apex and a large process oriented towards the molecular layer (Fig. 3A). 20.1% were constituted by similar triangular neurons whose base, however, faced towards the molecular layer. The latter characteristically gave rise to a descending process, often lining the wall of blood microvessels (Fig. 3B). A third type (15.1%) had a polygonal body that gave rise to variously oriented neuronal processes (Fig. 3C). The remaining 33.3% showed a roundish or

ovoid body closely adjacent to the wall of microvessels (Fig. 3D).

GAD 65/67-immunoreactive large neurons in the internal zone

A total of 79 GAD 65/67-immunoreactive large neurons (5.1%) were detected in this zone. The most common type (56.4%) was a neuron characterized by an ellipsoid, vertical body. It was observed only at the summit of the cerebellar folia and showed a positive axon-like process originating from the external pole of the body and directed towards the molecular layer (Fig. 4A). Another type (19.8%) was found in the deepest portion of the

Fig. 2A–E GAD 65/67-immunoreactive large neurons in the external zone of the granular layer. **A** A vertical, pear-shaped neuron showing immunoreactivity in the perikaryon and in apical processes (*arrowheads*) directed towards the molecular layer. **B** Another pear-shaped neuron whose body is closely adjacent to the body of a Purkinje neuron (P) that also appears to be immunoreactive. **C** An ovoid neuron and its positive axon-like process (*arrowheads*) among clusters of positive axon terminals and negative granule cells. **D** A horizontal, spindle-shaped neuron at the boundary between the granular and Purkinje neuron body layers; a positive dendrite-like process is seen to originate from one of the body opposite poles (*arrowhead*). **E** A positive neuron body (*arrow*) leaning against the wall of a microvessel (V). *Scale bars: A, C, D, 20 μm; B, E, 10 μm*



layer, sometimes intermingled with the nerve fiber bundles of the white matter of the cerebellar folia. This neuron type had a horizontal, pear-shaped body that gave rise to a positive axon-like process horizontally running at the boundary of the granular layer with the white matter, or intermingled with the nerve fibers of this latter (Fig. 4B, C). A third type (9.4%) had a polygonal body that gave rise to a number of variously oriented positive processes (Fig. 4D, E). Lastly, 14.4% were perivascular neurons, similar to those observed in the external and intermediate zones (Fig. 4F).

Discussion

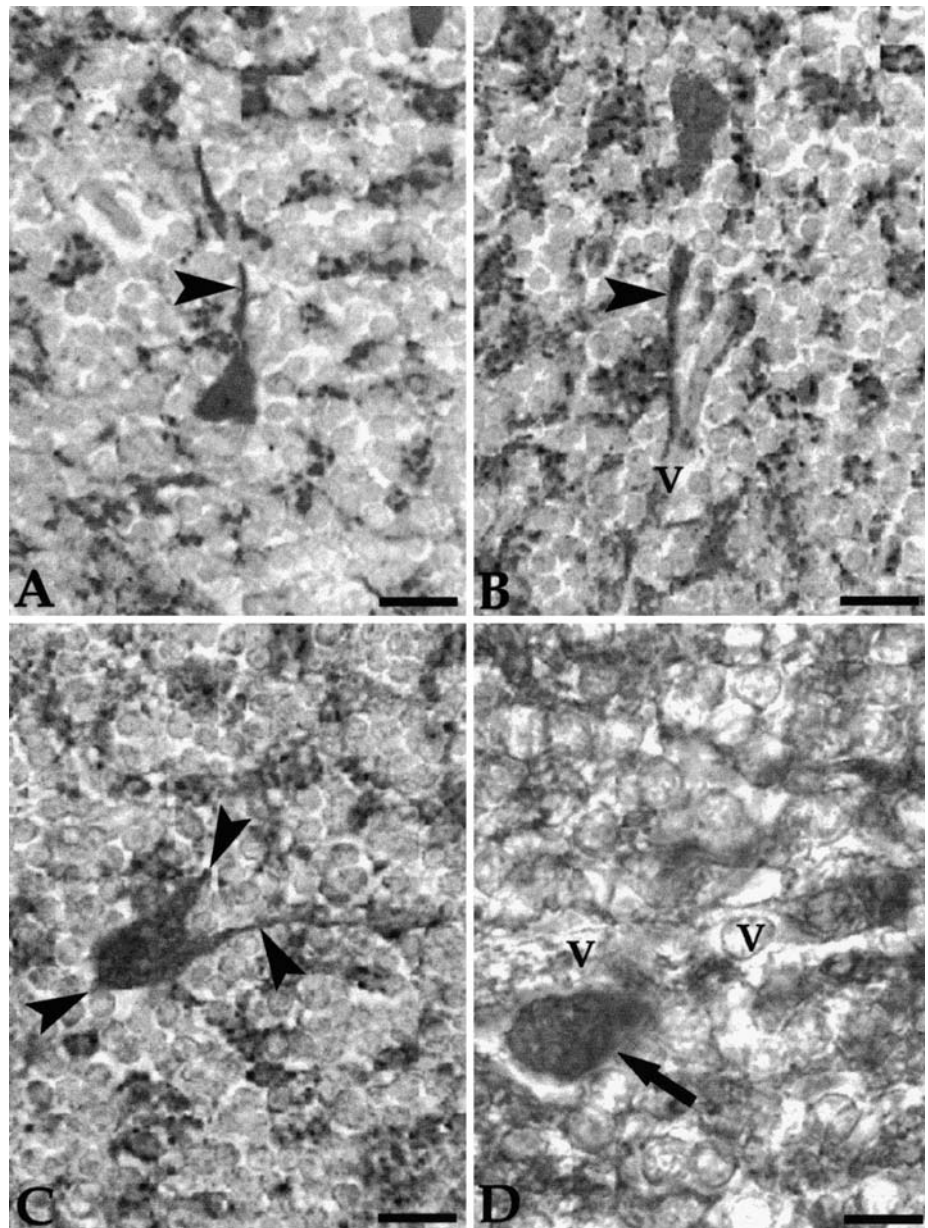
In a previous immunocytochemical study, carried out by means of the same antibody against both GAD 65 and GAD 67 we used in the present study, we demonstrated that GAD 65/67 antigenicity is preserved in postmortem human nerve tissues, where it maintains distribution patterns similar to those detectable in fresh tissues (Benagiano et al. 2000). On the basis of this finding, we searched the granular layer of the postmortem human cerebellar cortex in order to ascertain the presence,

quantify the distribution and identify the morphological types of the immunoreactive large neurons.

Observations carried out on all GAD-immunolabeled sections, as well as on neighboring toluidine blue-stained sections, indicated the presence of distinct types of GAD-immunoreactive large neurons distributed in all the cerebellar lobules examined and excluded the presence of GAD-immunonegative subpopulations among them.

Most of the immunoreactive large neurons were found in the external zone of the granular layer (83.7%). The neurons most frequently seen were vertical, pear-shaped neurons with their bodies squeezed against the bodies of Purkinje neurons or intercalated among them. The localization and shape of the body of this neuron type and the spatial orientation of its apical processes suggested that it corresponds to the candelabrum neuron. This neuron type has been described in the granular layer of the rat cerebellar cortex (Lainé and Axelrad 1994), but appears also similar to the 'intercalated' neuron, observed in the cat cerebellar cortex using silver impregnation techniques (Pensa 1931). The candelabrum neuron, which surprisingly was the large neuron type we most frequently encountered in our observations, has never been described in the human cerebellar cortex. Its GAD immunoreactivity is described herein for the first time.

Fig. 3A–D GAD 65/67-immunoreactive large neurons in the intermediate zone of the granular layer. **A, B** Triangular neurons with a positive process ascending (**A**, *arrowhead*) towards the molecular layer, or descending (**B**, *arrowhead*) into the deep granular layer and lining the wall of a microvessel (**V**). **C** A polygonal neuron with variously oriented positive processes (*arrowheads*). **D** An ovoid neuron (*arrow*) adjacent to the wall of a microvessel (**V**). Scale bars: **A, B, C**, 20 μm ; **D**, 10 μm



The large neuron type with a descending axon ending in the clusters of positive puncta, which our previous research indicated to correspond to glomerular synaptic complexes (Benagiano et al. 2001), was identified as the Golgi neuron. The GABAergic nature of the Golgi neuron has been ascertained in various mammalian species, including man (Gabbott et al. 1986; Benagiano et al. 2000, 2001).

The large neuron type characterized by a horizontal, spindle-shaped body localized immediately beneath the Purkinje neuron body layer was recognized as the intermediate neuron of Lugaro. The present, first demonstration of GAD immunoreactivity in the Lugaro neuron of the human cerebellar cortex agrees with the results of immunocytochemical studies carried out on the rat

cerebellar cortex (Aoki et al. 1986; Lainé and Axelrad 1998).

A fourth type of GAD-immunoreactive large neurons consisted of perivascular roundish ones. Neurons with such anatomical characteristics were found also in the intermediate and internal zones.

Of the GAD-immunoreactive large neurons seen in the granular layer, 11.2% were localized in the intermediate zone. Immunoreactivity was found in triangular and polygonal neuron types that showed morphological features (shape, size, location and orientation of the body) similar to those of some subtypes of non-traditional large neurons described by Braak and Braak (1983) and included in the type II and III, respectively, of their classification. The anatomical connections and the func-

Fig. 4A-F GAD 65/67-immunoreactive large neurons in the internal zone of the granular layer. **A** A vertical, ellipsoid neuron from whose body a positive, apical, axon-like process (*arrowhead*) originates, showing varicosities and directed towards the molecular layer. **B, C** Horizontal neurons (*arrows*) close to the bundles of positive fibers of the white matter (**B**) or intermingled among them (**C**). **D, E** Polygonal neurons with variously oriented processes (**D**, *arrowheads*); an intensely labeled process is seen directed towards the cerebellar surface (**E**, *arrowhead*). **F** A perivascular neuron with positive processes (*arrowheads*), which are detectable for long tracts along the wall of a microvessel (*V*). *Scale bars*: **A**, 40 μ m; **B, C, D**, 30 μ m; **E, F**, 20 μ m

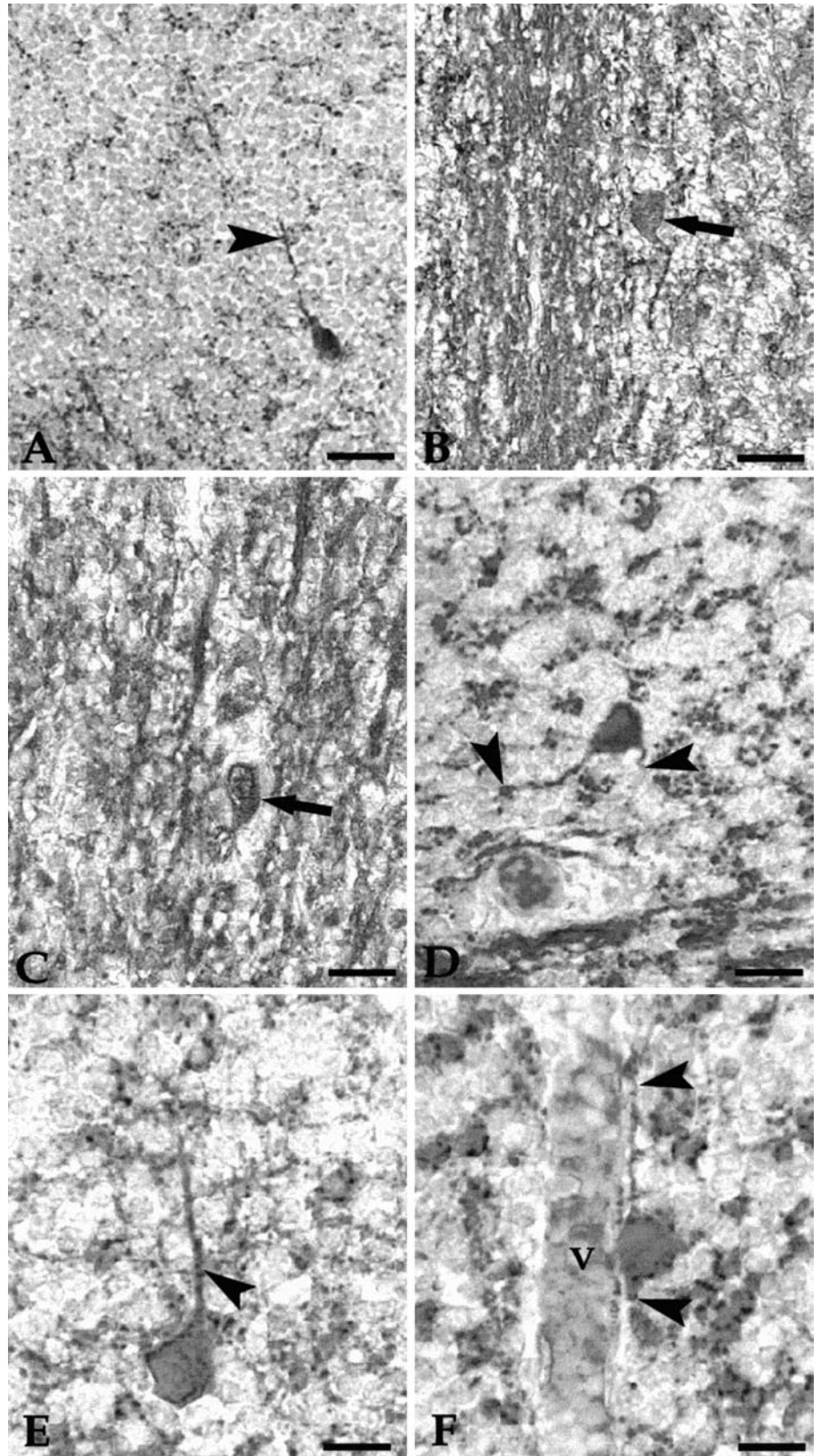


Table 1 Summary diagram of the main morphological features displayed by GAD 65/67-immunoreactive large neuron types in the granular layer of the human cerebellar cortex, and their relationship to neuronal types described in literature. The various types are numbered for each zone according to their density order, except the perivascular type, which is distributed throughout the layer

Large neuron type/distribution in the granular layer	Body form and orientation	Processes form and orientation	Interpretation based on comparison with literature data
Type 1/external zone	Pear-shaped, vertical with larger pole located internally	Dendrite-like and axon-like processes ascending from the external pole	Candelabrum neuron
Type 2/external zone	Polygonal/ovoid, vertical	Axon-like process from the internal portion of the body, confined in the granular layer	Golgi neuron
Type 3/external zone	Spindle-shaped, horizontal	Dendrite-like processes from body opposite poles, running horizontally along the boundary with the Purkinje neuron body layer	Lugaro neuron
Type 1/intermediate zone	Triangular, vertical with apex located externally	Ascending process directed towards the molecular layer	A subtype of Braak and Braak type II
Type 2/intermediate zone	Triangular, vertical with apex located internally	Descending process, often in an anatomical relationship with microvessels	A subtype of Braak and Braak type II
Type 3/intermediate zone	Polygonal	Variouly oriented processes	A subtype of Braak and Braak type III
Type 1/internal zone	Ellipsoid, vertical	Axon-like process from the external pole, directed towards the molecular layer	A subtype of Braak and Braak type II
Type 2/internal zone	Pear-shaped, horizontal	Axon-like process horizontally running along the boundary with, or inside, the white matter	Synarmotic neuron
Type 3/internal zone	Polygonal	Variouly oriented processes	Globular neuron
Perivascular type/external-intermediate-internal zones	Roundish/ovoid	Perivascular processes	-

tional roles played by these GAD-immunoreactive large neurons in the cerebellar circuitry remain unclear.

GAD-immunoreactive large neurons were poorly represented (5.1%) in the internal zone of the granular layer. A first neuron type with an ellipsoid, vertical body, located at the summit of the cerebellar folia, closely resembled a subtype of the neuron type II already described in the human cerebellum (Braak and Braak 1983).

The second type was a horizontal neuron found at the boundary between the cortex and the white matter or intermingled among the nerve fibers of the white matter, which was identified as the synarmotic neuron on the basis of the course of its axon-like process, parallel to the nerve fiber bundles of the white matter. Although the synarmotic neuron was one of the first 'non-traditional' neurons to be described in the human cerebellar cortex (Landau 1933), it is still relatively unknown and evidence of its putative GABAergic nature is supplied here for the first time.

Lastly, the polygonal neuron type observed in the internal zone appeared similar to the globular neuron, a large neuron recently described in the rat cerebellar cortex by silver impregnation techniques and immunocytochemistry for calretinin (Lainé and Axelrad 2002).

The unipolar brush neuron, which has been generally dealt with in the introduction, is not mentioned here, because it was never observed in our sections immunocytochemically tested for GAD. This is in agreement with data from literature indicating a possible glutamatergic nature of the unipolar brush neuron (Nunzi et al 2001), as

well as its preferential distribution in the flocculonodular lobe (not examined in the present study) (Mugnaini and Floris 1994).

A summary diagram displaying the various neuron types distinguished in the present text and the relationships between these types and those of the large neuron types described in literature is supplied in Table 1.

The large neurons of the granular layer revealed by GAD 65/67 immunoreactions can mainly be considered as interneurons involved in intrinsic, non-projective circuits of the cerebellar cortex. The Golgi neuron, owing to its GABAergic inhibiting nature, may be involved in the modulation of the activities of the cerebellar glomeruli, where afferences arrive vehicled by glutamatergic, excitatory mossy fibers (Storm-Mathisen et al. 1983; Clements et al. 1987). On the other hand, the GABAergic, inhibiting Lugaro and candelabrum neurons may be involved, in view of their projections to the molecular layer, in the modulation of the activities of GABAergic, inhibiting stellate and basket neurons (Lainé and Axelrad 1994, 1998). This can result in either inhibition of an excitatory impulse, or inhibition of an inhibiting impulse, i.e. disinhibition.

GAD 65/67 immunoreaction also revealed the synarmotic neuron, which would be involved in different types of cerebellar cortex circuits (Jansen and Brodal 1958). In fact, the axon of this neuron, directed towards the white matter, may cross the white matter to reach the cortex of the other surface of the same folium, or run from one cerebellar folium to another. Consequently, an associative function between different regions of the cerebellar cortex

could be attributed to this neuron. Moreover, the synarmotic neuron could also be interpreted as a projective neuron, involved in extrinsic corticocerebellar circuits, constituting a second source of GABA-mediated outputs from the cerebellar cortex, besides the Purkinje neuron. The existence of a second type of projective neuron, probably corresponding to the synarmotic neuron, has been postulated in the mouse cerebellar cortex (Müller 1994).

Another important aspect of the GABA functions concerns its role, among those of other neurotransmitters/neuromodulators, in the regulation of the circulation and permeability of the intrinsic microvessels within the central nervous system (CNS). The anatomical basis of this vasoregulatory function is constituted by GABA-containing axon terminals that synapse with components of CNS microvessel walls (Gragera et al. 1993; Benagiano et al. 2001). The immunoreactive triangular neuron of the intermediate zone that projects its axon-like process towards microvessel walls could be the source of GABA-containing terminals involved in this nervous regulation as regards the cerebellar cortex.

Finally, there are GAD 65/67-immunoreactive large neurons, distributed in each zone of the granular layer of all lobules examined, whose bodies we saw lying extensively on the microvessel walls: they could represent a new type of 'non-traditional' neuron of the granular layer and could intervene in the local regulation of the microvessel functions, e.g. by means of an extrasynaptic release of GABA in the perivascular microenvironment, or, in other words, through a volume transmission mechanism.

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