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An Immunofluorescence Study About Staining Pattern Variability of Sarcoglycans in Rat's Cerebral and Cerebellar Cortex

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Abstract

Title: An Immunofluorescence Study About Staining Pattern Variability of Sarcoglycans in Rat's Cerebral and Cerebellar Cortex.

Background: Sarcoglycans are transmembrane glycoproteins which connect extracellular matrix components to cytoskeleton. This protein system has been long studied in muscle but there are few data about its localization in non-muscular tissues.

Methods and Findings: In the present report, we have conducted an indirect immunofluorescence study on normal rat's cerebral and cerebellar cortex. Our results show that in these districts each sarcoglycan is expressed by a "spot-like" staining pattern, with spots of 0.5-2 µm average diameter, extending mainly around the soma of neurons and glial cells. In cerebral cortex, although all sarcoglycans are present, a staining pattern variability for each sarcoglycan, in the different cerebral cortex areas, exists. Instead, the pattern distribution level of sarcoglycans in cerebellar cortex doesn't change. We also performed a statistical analysis which confirms the immunofluorescence results.

Conclusions: Then, the presence of a sarcoglycans variability in cerebral cortex, where it is known the existence of several synaptic network, and the absence of a sarcoglycans variability in cerebellar cortex, where the same synaptic networks are repeated unchanged, suggest that in brain sarcoglycans may be associated with synaptic networks. Moreover, the distribution of sarcoglycans, mainly in post-synaptic regions of the neurons, suggests a role of these proteins in cellular signalling, regulating membrane receptors assembly. We also support that sarcoglycans in glial cells could be associated with the regulation of the mechanism in the brain-blood-barrier.

Keywords: Sarcoglycans; Cerebral cortex; Cerebellar cortex; Neurons; Rat

Introduction

The Sarcoglycans Sub-Complex (SGC) is a member of Dystrophin-Glycoprotein Complex (DGC) which also includes a dystroglycan sub-complex, made up of α- and β-dystroglycans, and a sarcoplasmic sub-complex made up of dystrophin, dystrobrevins, syntrophins and nNos.

The SGC, which is fixed to the dystrophin axis by a lateral association with the dystroglycan sub-complex [1], is made up of four transmembrane glycoproteins: α-, β-, γ- and δ-sarcoglycans. These glycoproteins present a short intracellular domain, a single transmembrane domain and a large extracellular domain with a carboxyl-terminal cluster, rich in several cysteine residues [2]. In particular, α-sarcoglycan (50kD) is considered a type I protein, harbouring the N-terminal on the extracellular side; β-, γ- and δ-sarcoglycans (43kD, 35kD and 35kD respectively) are considered type II proteins, harbouring the N-terminal on intracellular side [3]. In fact, immunoprecipitation and cross linking investigation suggest that β-, γ- and δ-sarcoglycans are tightly associated to each other to form a functional unit [4,5]. In contrast, α-sarcoglycan can be dissociated from the complex under relatively mild conditions [4]. This suggests that the sarcoglycans may form two different subunits: one subunit made up of α-sarcoglycan and one subunit made up of β-, γ- and δ-sarcoglycans [4,5]. Molecular investigations have shown the assembly of the sarcoglycans components: the first step is the forming of a central core, made up of β- and δ-sarcoglycans; in a second step α-sarcoglycan links the β/δ-core and than γ-sarcoglycan determinate the maturation of the complex and the linkage to dystrophin [6,7].

A fifth sarcoglycan, ε-sarcoglycan, highly homologous to α-sarcoglycan, has been identified [8]. It is widely expressed in

most tissues and so is not considered muscle-specific [4,8,9]. After identification of ε-sarcoglycan the study of sarcoglycans in muscle was extended also in smooth muscle fibers hypothesizing that ε-sarcoglycan might replace α-sarcoglycan [10].

A novel sarcoglycan, ζ-sarcoglycan, has been identified; this glycoprotein present high homologies with γ- and δ-sarcoglycan [11]. The identification of two novel isoforms suggests that these proteins link to each other to form two different tetrameric complexes: one complex, made up of α-, β-, γ- and δ-sarcoglycans, which is characteristic of cardiac and skeletal muscle but it is absent in smooth muscle, and a second complex, made up of β-, δ-, ε- and ζ-sarcoglycans, which is present in all of three types of muscular tissues [12]. Although that, our previous study on smooth muscle have shown the presence of all sarcoglycans, suggesting the possibility of the existence of a pentameric or hexameric complex with a higher or lower expression of individual isoform depending on muscle type [13].

Sarcoglycans have been long studied, such as dystrophin, because of their involvement in some forms of hereditary muscular dystrophy as sarcoglycanopathy or Limb Girdle Muscular Dystrophy (LGMD) [2,14-16]. For these reasons, this sub-complex has been mainly studied in muscular tissues and there are few data about SGC in non-muscular tissues. The study of sarcoglycans in non-muscular tissues has been mainly conducted in Central Nervous System (CNS) where recent researches have shown the importance of ε-sarcoglycan because its mutation determines Myoclonus Dystonia Syndrome (MDS) [17]. Because of its involvement in this pathology of CNS, ε-sarcoglycan have been investigate in several regions of the brain as cerebral and cerebellar cortex [18]; moreover, it was also demonstrated that ζ-sarcoglycan is also highly expressed in brain [19]. Although two isoforms of SGC have been found in CNS, other isoforms have never been tested in brain. Furthermore, it was hypothesized the existence of a "DGC-like" characteristic of the brain, involved in postsynaptic clustering and stabilization of some inhibitory GABAergic synapses, which is made up of the entire dystroglycan sub-complex and the entire sarcoplasmic sub-complex whereas the sarcoglycan sub-complex is formed only by ε- and ζ-sarcoglycan nevertheless, for this author, although theoretically possible, the existence of a prototypical tetrameric sarcoglycan complex in brain is unlikely [20]. Instead, an our recent report, has demonstrated the expression of α-, β-, γ-, δ- and ε-sarcoglycans in human cerebral cortex [21] demonstrating a difference between molecular results and immunofluorescence results when the biopsies have not been extracted from same region of the brain [21]. On this basis, in the present report, by immunofluorescence, we verify the presence of sarcoglycans in rat's cerebral and cerebellar cortex; moreover, we verify if these proteins show an uniformly expression or if a different distribution pattern among the cerebral cortex areas and in cerebellar cortex exists.

Materials and Methods

Immunofluorescence

In the present study, normal male adult Wistar rat was used. The work described has been carried out in accordance with EU Directive 2010/63/EU for animal experiments.

The animal was sacrificed after anaesthesia and than its cerebrum and cerebellum were extracted and were fixed in 3% paraformaldehyde in 0.2 mol/L phosphate buffer, pH 7.4. After numerous rinses in 0.2 mol/L phosphate buffer and Phosphate Buffered Saline, 0.2 mol/L, pH 7.6 with 0.9% NaCl (PBS), the cerebrum and cerebellum were infiltrated with 12% and 18% saccharose and than they were divided in two hemispheres that were frozen in liquid nitrogen and were stored at -20°C. By cryotomy, the hemispheres have been cut with coronal cuts, in anterior to posterior direction, in 30 µm sections collected on glass slides coated with 0.5% gelatine and 0.005% chromium potassium sulphate. We have numbered all sections and we have chosen for the cerebrum 20 anterior sections, corresponding to frontal-parietal regions and 20 posterior sections, corresponding to parietal-temporal regions; for the cerebellum we have chosen the first 20 anterior and the last 20 posterior sections. On these sections four series of indirect immunofluorescence reactions were performed: 1) single localization for α-, β-, γ-, δ-, ε- and ζ-sarcoglycans both in cerebrum and cerebellum; 2) double localization between each sarcoglycan and SMI-32, a protein which marks a non-phosphorylated epitope of neurofilament proteins of neurons in cerebrum; 3) double localization between each sarcoglycan and calbindin, a protein which marks the calcium channel activity in neuronal cells in cerebellum; 4) double localization between each sarcoglycan and GFAP, a protein which marks the green fluorescent acid glial cells both in cerebrum and cerebellum.

To block non-specific sites and to make the membranes permeable, the sections were pre-incubated with 1% bovine serum albumin (BSA) and 0.3% Triton X-100 in PBS at room temperature for 15 min. Finally, the sections were incubated primary antibodies at room temperature for 2h. The following primary antibodies obtained from Santa Cruz Biotechnology, Inc., California, were used: goat polyclonal anti-α-sarcoglycan (diluted 1:100); goat polyclonal anti-β-sarcoglycan (diluted 1:100); goat polyclonal anti-γ-sarcoglycan (diluted 1:100); goat polyclonal anti-δ-sarcoglycan (diluted 1:100); goat polyclonal anti-ε-sarcoglycan (diluted 1:100); goat polyclonal anti-ζ-sarcoglycan (diluted 1:100). All primary antibodies were demonstrated with Texas-Red-conjugated IgG anti goat (1:100 dilution; Jackson ImmunoResearch Laboratories, West Grove, Pa). The fluorochrome-labelled antibody was applied for 1h at room temperature. For the double localization reactions, after incubation with the secondary antibodies and after three rinses of 10 min each one, the sections were incubated also with the following primary antibodies at room temperature: mouse monoclonal anti-SMI-32RT (1:1000 dilution; COVANCE, Emeryville, California); mouse monoclonal anti-calbindin D28K (1:100 dilution; Santa Cruz Biotechnology Inc., California); mouse monoclonal anti-GFAP (1:100 dilution; Santa Cruz Biotechnology Inc., California). These antibodies were

demonstrated with FITC-labelled anti-mouse antibody (1:100 dilution; Jackson ImmunoResearch Laboratories, West Grove, Pa). Than, to label nuclei the sections were incubated with DAPI (1:50 dilution; Sigma Aldrich, St. Louis, USA) for 10 min at room temperature. Finally, the slides were washes in PBS and sealed with mounting medium.

Two of the 20 anterior and two of the 20 posterior sections were used for negative control making the reaction avoiding the primary antibodies.

In order to verify the behaviour of SGC in rat's cerebral and cerebellar cortex, in each single sections we observed the sarcoglycans staining pattern in three different virtual areas: A, B and C areas (**Figure 1A and 1B**). Samples were observed with a Zeiss LSM 510 confocal microscope equipped with Argon laser (458 nm and 488 nm λ) and two HeNe laser (543 nm and 633 nm λ). All images were digitized at a resolution of 8 bits into an array of 2048 × 2048 pixels. Optical sections of fluorescence specimens were obtained at 488 nm λ, at 62/s scanning shipped with up to 8 on average. The pinhole was set for optimal resolution. Contrast and brightness were established by examining the most brightly labelled pixels and choosing the settings that allowed clear visualization of the structural details while keeping the pixel intensity at its highest (~200. Digital images were cropped and the figure montage prepared using Adobe Photoshop 7.0.

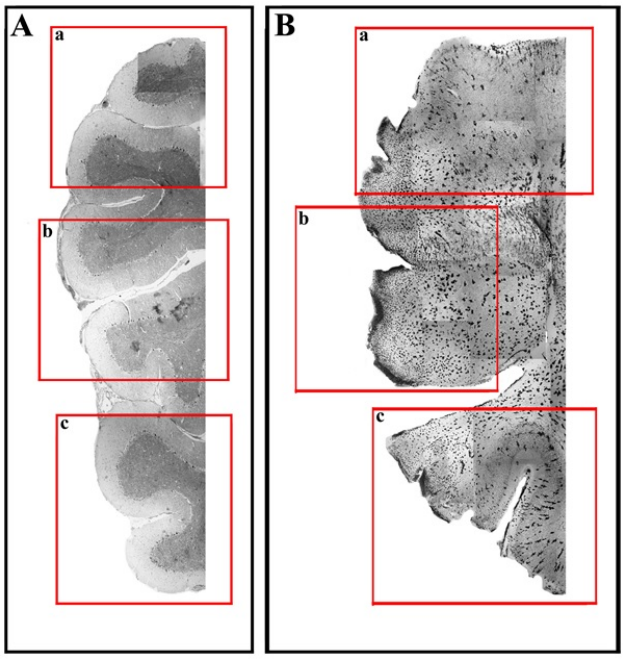

Figure 1. Rat's cerebral cortex section stained by Golgi-Cox impregnation technique (A) and rat's cerebellar cortex section stained by hematoxylin-eosin method (B). In both images, the A, B and C areas define the regions where the immunofluorescence reactions were observed.

The data, obtained with the observations of the sections both of cerebral and cerebellar cortex, are presented as frequencies α, β, γ, δ, ε and ζ-saroglycan. Comparisons between proportions of discreet variables was performed using chi-square and Cramer's V test. The level of statistical significance was always set at P<0,05. Cramer's V value close to 0 shows little association between variables.

Golgi–Cox impregnation

Immediately after decapitation, the cerebrum was incubated in a Golgi–Cox solution (5% K_2CrO_4, 5% HgCl, and 5% $K_2Cr_2O_7$) for 28 d, after which it was imbedded in celloidine and, with a microtome, it was cut into 200-µm-thick sections.

Haematoxylin-Eosin stain

The cerebellum was fixed overnight in 2.5% glutaraldehyde plus 4% paraformaldehyde in 0.1 M sodium cacodylate buffer at 4°C, dehydrated in ethanol and infiltrated with Technovit 9100. Sections of 7-µm thickness were cut with an -microtome and were counterstained with haematoxylin and eosin.

Both the sections of cerebrum and cerebellum were observed and photographed with an Olympus BH-2 microscope. The sections were obtained by a reconstruction using Adobe Photoshop 7.0 and converted in black and white colours.

Results

Cerebral cortex

Immunofluorescence: The results of single localization reactions show that α-, β-, γ-, δ-, ε- and ζ-sarcoglycans are expressed in rat's cerebral cortex (**Figure 2**). They present a "spot-like" staining pattern with spots of 0.5-2 µm average diameter, arranged mainly around cellular soma (**Figure 3**).

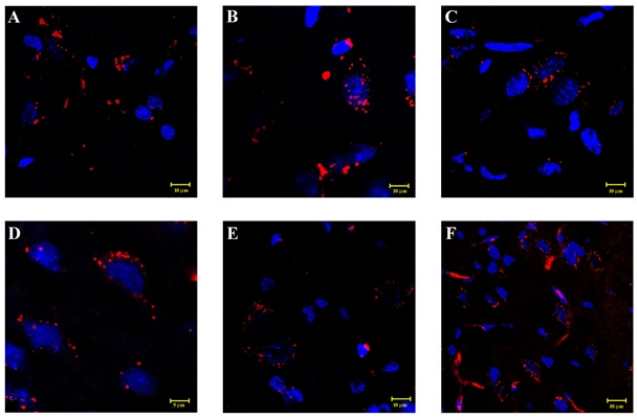

Figure 2. Compound panel of immunofluorescence findings in rat's cerebral cortex. All samples were immunolabelled with antibodies against α-SG (A), β-SG (B), γ-SG (C), δ-SG (D), ε-SG (E) and ζ-SG (F) (red channel). The blue channel represents nuclear-staining DAPI.

The double localization reaction between each sarcoglycan and SMI-32 shows that sarcoglycans are expressed around the soma of pyramidal cells (**Figure 4A**) and the double localization between each sarcoglycan and GFAP show that sarcoglycans are

also expressed in glial cells (**Figure 4B**). Though all of sarcoglycans are present in cerebral cortex, each isoform is not always expressed in all observed cells; in fact, among the A, B and C areas of a same section and between the A, B and C areas of anterior sections and the A, B and C areas of posterior sections the level of each sarcoglycan around cellular soma changes.

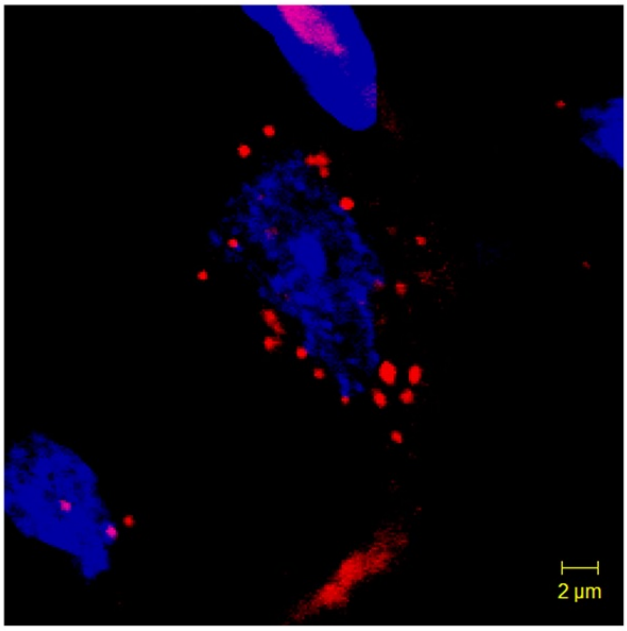

Figure 3. High magnification which shows the spot-like staining pattern of α-SG around the soma of the cells (red channel), with spot of 0,5-2-µm average diameter; the blue channel represents nuclear-staining DAPI. This characteristic staining pattern was found for each sarcoglycan (data not shown).

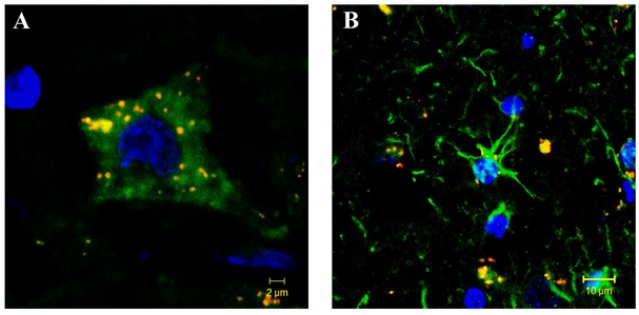

Figure 4. Immunofluorescence findings on rat's cerebral cortex, showing β-SG staining pattern (red channel) localized in pyramidal neurons, marked with SMI-32 (green channel). Also in the glial cells, marked with GFAP (green channel), the staining pattern for γ-SG was present (b). In both images, the blue channel represents nuclear-staining DAPI; the yellow fluorescence represents a merge between red and green channels. The same data were found for each sarcoglycan (data not shown).

Statistical analysis: To establish with a statistical evaluation the difference in sarcoglycans level in the cerebral cortex, we have numbered the red spots laid out around 100 cells in A, 100 cells in B and 100 cells in C areas both of anterior and posterior sections; this evaluation was performed for each sarcoglycans. The 100 cells have been chosen by random way and we numbered only the spots which weren't less than 0.5 µm and more than 2 µm to avoid wrong positivity. In A area of anterior sections, α- and δ-sarcoglycans are less expressed than other sarcoglycans, with 33 and 79 spots respectively; in B area, α- and γ-sarcoglycans are less expressed than other proteins with 22 and 48 spots respectively; in C area, α-sarcoglycan is much less expressed than other sarcoglycans, with 55 spots (**Table 1**).

Table 1. Count of the spots in rat's cerebral cortex.

	Anterior sections			Posterior sections		
	Areas			Areas		
	A	B	C	A	B	C
α	33	48	55	112	114	89
β	139	123	129	5	12	12
γ	134	22	205	270	213	158
δ	79	153	293	171	191	91
ε	221	124	280	240	220	164
ζ	110	100	115	135	126	200

In A area of posterior sections, β-sarcoglycan is much less expressed than other sarcoglycans with 5 spots; in B area, β-sarcoglycan continues to be much less expressed than other glycoproteins with 12 spots, in C area, all of sarcoglycans are less expressed than in A and B areas (**Table 1**). Comparing the results of anterior and posterior sections, it's clear that α-, β- and γ-sarcoglycans are the most variable; in fact, α-sarcoglycan changes from low levels in anterior sections (33 spots A; 48 spots B; 55 spots C) to high levels in posterior sections (112 spots A; 114 spots B; 89 spots C). Even β-sarcoglycan changes, from high levels in anterior sections (139 spots A; 123 spots B; 129 spots C) to low levels in posterior sections (5 spots A; 12 spots B; 12 spots C). Moreover, there is a lot of difference in γ-sarcoglycan level between B area of anterior sections (22 spots) and B area of posterior sections (213 spots). All of these results were used to perform the Chi-square and Cramer's V test which have shown that a statistical significant variability of sarcoglycans staining pattern among the A, B and C areas both in anterior sections and in posterior sections and between the three areas of anterior sections and the three areas of posterior sections (**Table 2**).

Table 2. Chi-square and Cramer's V test results of cerebral cortex count.

Anterior sections			Posterior sections		
Areas			Areas		
A	B	C	A	B	C

Chi-square=168.32	Chi-square=21.93
(P=0.0001)	(P=0.005)
Cramer's V=0.203	Cramer's V=0.072

Anterior sections vs Posterior sections		
A ant vs A post	**B ant vs B post**	**C ant vs C post**
Chi-square=226.13 (P=0.0001)	Chi-square=253.4 (P=0.0001)	Chi-square=123.13 (P=0.0001)
Cramer's V=0.4013	Cramer's V=0.455	Cramer's V=0.288

Cerebellar cortex

Immunofluorescence: The results of single localization reactions show that α-, β-, γ-, δ-, ε- and ζ-sarcoglycans are expressed in rat's cerebellar cortex (**Figure 5**). These results also show that the sarcoglycans pattern is concentrated mainly in a single cell layer which probably correspond to the Purkinje cell layer. The double localization reactions between each sarcoglycans and calbindin confirm that sarcoglycans are mainly laid out around the soma of Purkinje cells (**Figure 6A**); sarcoglycans are expressed also in glial cells (**Figure 6B**). Around the soma of the Purkinje cells sarcoglycans present the same "spot-like" staining pattern with spots of 0.5-2 µm average diameter (**Figure 7**). Although that, some positivity of sarcoglycans is detectable also in granular layer and in molecular layer, where they have also shown a linear staining pattern (yellow arrow) (**Figure 8**). The observation in A, B and C areas of anterior and posterior sections of the cerebellar cortex have not shown a variability in sarcoglycans expression but each glycoprotein seem to be present at the same level in all of the observed cerebellar cortex regions.

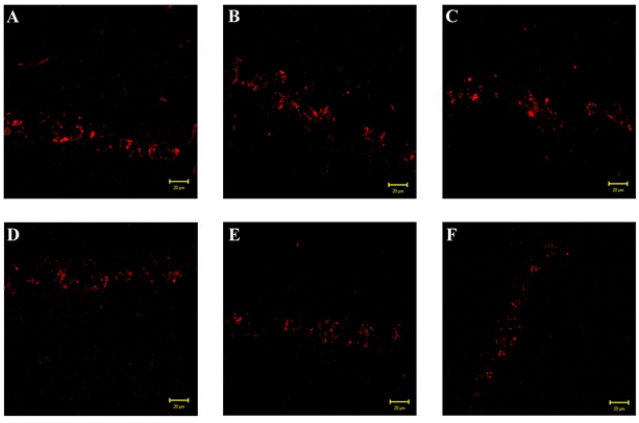

Figure 5. Compound panel of immunofluorescence findings in rat's cerebellar cortex. All samples were immunolabelled with antibodies against α-SG (A), β-SG (B), γ-SG (C), δ-SG (D), ε-SG (E) and ζ-SG (F) (red channel). All tested proteins were present in this tissue.

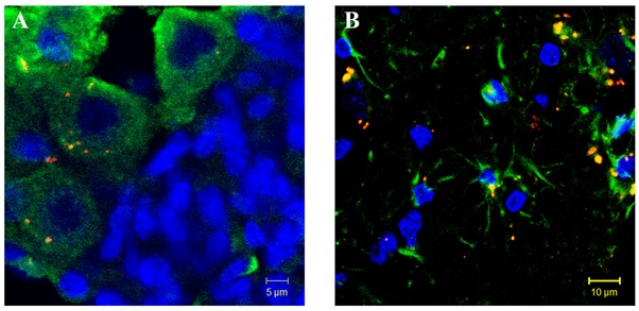

Figure 6. Immunofluorescence findings showing the ε-SG staining pattern (red channel) in the Purkinje cells marked with calbindin (green channel) (A) and the δ-SG (red channel) expressed in glial cells marked with GFAP (green channel) B). In both images the blue channel represents nuclear-staining DAPI, the yellow fluorescence represents a merge between red and green channels. Also other sarcoglycans show same behaviour (data not shown).

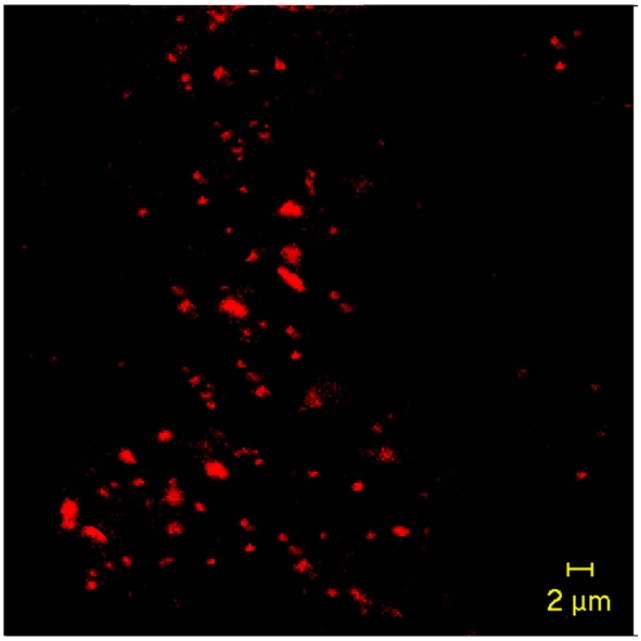

Figure 7. High magnification showing the spot-like staining pattern for ζ-SG around the soma of the cells in the rat's cerebellar cortex (red fluorescence), with spot of 0,5-2-µm average diameter. These characteristic staining pattern was found for each sarcoglycan.

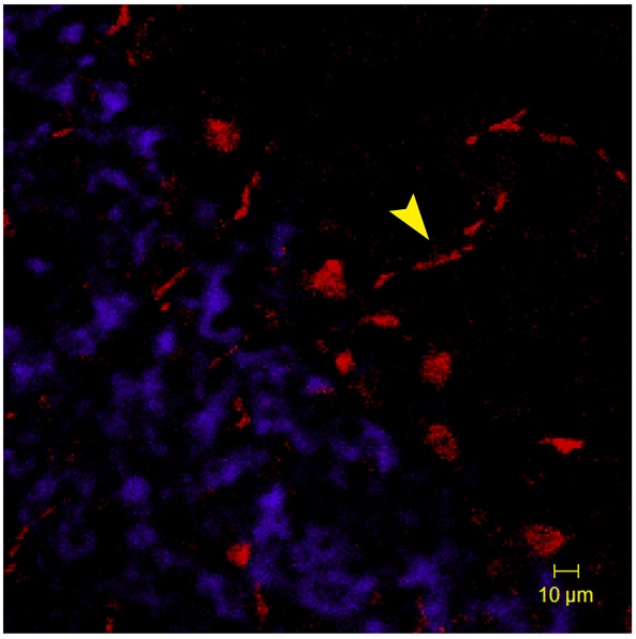

Figure 8. Immunofluorescence results showing a linear staining pattern for α-SG, in rat's cerebellar cortex (red channel), probably corresponding to a Purkinje dendritic extensions within the molecular layer (yellow arrow). The blue channel represents nuclear-staining DAPI.

Statistical analysis: In cerebellar cortex, we performed the count of the spots around 100 cells in A, 100 cells in B and 100 cells in C areas both of anterior and posterior sections. In A, B and C areas of anterior sections all sarcoglycans show values which are very similar to each other with a minimum number of 400 spots for β-sarcoglycan in B area and a maximum number of 530 spots for the ε-sarcoglycan both in B and C areas (**Table 3**). Moreover in posterior sections, the numbers of the spots for each sarcoglycan in the three observed areas are similar to each other with a minimum number of 405 for the β-sarcoglycan and a maximum number of 520 both for γ- and ε-sarcoglycans in B and A areas, respectively (**Table 3**). Although β-sarcoglycan seems to be the less expressed, ε-sarcoglycan appears the most expressed; the results of the Chi-square and Cramer's V test confirm the immunofluorescence results, showing the absence of a significant difference in the sarcoglycans pattern distribution among the A, B and C areas both of anterior and posterior sections and between the three areas of anterior and posterior sections (**Table 4**).

Table 3. Count of the spots in rat's cerebellar cortex.

	Anterior sections			Posterior sections		
	Areas			Areas		
	A	B	C	A	B	C
α	426	410	430	435	408	432
β	408	400	420	410	405	417
γ	520	522	515	510	520	505
δ	480	470	460	480	463	468
ε	525	530	530	520	516	500
ζ	500	510	490	505	499	500

Table 4. Chi-square and Cramer's V test results of cerebellar cortex count.

Anterior sections			Posterior sections		
Areas			Areas		
A	B	C	A	B	C
Chi-square=0.22			Chi-square=0.21		
(P=0.99)			(P=0.99)		
Cramer's V=0.0068			Cramer's V=0.0067		
Anterior sections vs Posterior sections					
A ant vs A post		B ant vs B post		C ant vs C post	
Chi-square=0.22		Chi-square=0.19		Chi-square=0.82	
(P=0.99)		(P=0.99)		(P=0.93)	
Cramer's V=0.0068		Cramer's V=0.0064		Cramer's V=0.0132	

Discussion

In CNS a dystrophin-glycoprotein complex exists but differs in composition and function from the DGC core present in muscle due to presence of several dystrophin isoforms and to existence of a sarcoglycan complex made up only of ε- and ζ-sarcoglycans; for these reasons it was called "DGC-like" [20]. In fact, the DMD gene encodes several isoforms of dystrophin, most of which has been found in CNS and in peripheral nervous system [22]. The main difference in the DGC-like from the classical DGC is the presence of an incomplete sarcoglycan sub-complex for the absence of the α-, β-, γ- and δ-sarcoglycans which have never been investigated in CNS yet. The ε-sarcoglycan was the most investigated in brain because it was demonstrated that a mutation of this isoform is responsible of Myoclonus Dystonia Syndrome (MDS) [17]; different studies have shown that this protein is present in many brain regions [18,23] suggesting a key role of this glycoprotein in CNS. The literature show that also ζ-sarcoglycan is highly expressed in brain but it was demonstrated only using molecular techniques [19] and its function is still unclear. The present report has shown that in rat's cerebral and cerebellar cortex, besides ε- and ζ-sarcoglycans, the α-, β-, γ- and δ-sarcoglycans are present too. These results showing the presence of sarcoglycans in rat's brain, are in accordance with an our previous report which has demonstrated the presence of all sarcoglycans in human intrasurgical biopsies of cerebral cortex, both in neuronal and glial cells [21]. Different report show that ε-sarcoglycan is present around pyramidal cells of different brain regions and in particular it is around the soma of dopaminergic (DAergic) and serotonergic (5-HTergic) cell groups; in the cerebellum, ε-sarcoglycan has been found around in the Purkinje cell layer and molecular layer, but it has been found absent in the granular layer [18]. Molecular and immunofluorescence results of the present report show that all sarcoglycans are

present both in rat's cerebral and cerebellar cortex. In particular, in cerebral cortex each tested sarcoglycan is expressed, by a "spot-like" staining pattern, around the soma of pyramidal cells, glial cells and around the soma of cells that are likely to be granular neurons or interneurons. In cerebellar cortex we have found that sarcoglycans are expressed by a "spot-like" staining pattern mainly around the soma of the Purkinje cells and also around the soma of glial cells of the granular and the molecular layer; in the granular and molecular layer we also found cells which were negative for calbindin and GFAP but which were positive for sarcoglycans and they are likely to correspond to Golgi interneurons for the granular layer and basket and stellate cells for the molecular layer. Moreover, in cerebellar cortex sarcoglycans also present a linear staining pattern in granular and molecular layer that could mark dendritic extension. All these findings, despite the evidence of a tetramer arrangement of SGC [23-25], suggest strongly that the same pentameric/ exameric arrangement of sarcoglycans that we have hypothesized to be present in muscle [13] it could be also present in brain where this complex may play a different role from the role played in muscle.

About the sarcoglycans function in CNS, a correlation between ε-sarcoglycan and synaptic network it was demonstrated; in fact some studies have supported that this sarcoglycan is associated with dopaminergic neurons in substantia nigra and retinal tegmental area; moreover, preliminary neurochemical analysis of ε-sarcoglycan- deficient mice showed increased levels of dopamine and its metabolites within the striatum [26,27]. Besides, it was hypothesized that the dystrophin and then the DGC are involved in modulation synapse function at a subset of GABAergic synapses in the hippocampus and cerebellum [28,29] and also dystroglycans and dystrobrevins are correlated with GABAergic receptors in cerebellum [30]. Sarcoglycans were also found in Schwann cells of peripheral nervous system where they are involved in myelin stabilization [31]; DGC has been found to be expressed on astrocyte end-feet and it has been hypothesized that it might play a role in the regulation of transport mechanism across the blood-brain barrier [32].

By a spot count method, supported by a statistical analysis, we have found that in rat's cerebral cortex sarcoglycans present a staining pattern variability with significant differences in number of spots between front-parietal and parietal-temporal regions and among the different areas of a single sections. Instead, in cerebellar cortex, we have not found a variability in sarcoglycans staining pattern; in fact, there were no significant differences in number of spots for each isoforms between anterior and posterior regions and among the different areas of a single section and the staining pattern level of all glycoproteins has shown to be similar to each other; moreover, results have shown that in cerebellar cortex there was a bigger amount of spots for cell than cerebral cortex. It is well known that the different areas of cerebral cortex play a specific role using various kind of synaptic network to do their functions [33]; therefore, we can hypothesize that the staining pattern variability of each sarcoglycans may be correlated with the variability of synaptic networks, characteristic of cerebral cortex. The absence of variability of sarcoglycans in cerebellar cortex is in accordance to the its structure that presents the same three layers (molecular, granular and gangliar) in every areas which discharge one's duties using the same synaptic networks; that supports the of a correlation between SGs and synaptic network in brain.

In our opinion in cerebral cortex, because sarcoglycans are mainly expressed in post-synaptic sites, all sarcoglycans could be involved in dopaminergic or GABAergic neurotransmission or they could be involved in other type of synaptic network in dependence of the cerebral cortex area where they are present; in cerebellar cortex the big amount of sarcoglycans around the soma of Purkinje cells and the presence also around the interneurons and along the dendritic extension suggest us that they could be predominantly associated with GABAergic receptors which are the main representative of the cerebellar neurotransmission. In fact, our preliminary results of rat's cerebral and cerebellar cortex have shown a colocalization between each sarcoglycan and GABAergic receptor in brain (data no shown). These results, show the important evidence that the entire SGC could plays a key role in brain, representing a specific complex, present in the cerebral cortex, which has alternative and/or additional functions independent of the conventional sarcoglycan complex.

These results open a new research line concerning the study of sarcoglycans in non-muscular tissues, demonstrating again that these proteins are no muscular specific. Furthermore, because most studies suggested the presence of a bidirectional signalling between SGs and integrins both in striated and in smooth muscle [13,34-38], an intriguing challenge in this area of research could be verify the presence of brain integrins and to understand how these proteins could interact with sarcoglycans in the different areas of cerebral cortex and in cerebellar cortex.

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