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Comparing Astrocytic Gap Junction of Genetic Absence Epileptic Rats with Control Rats: An Experimental Study

Safiye Cavdar (scavdar@ku.edu.tr)
Koc Universitesi
Büşra Köse
Koc University: Koc Universitesi
İlknur Sur
Koc University: Koc Universitesi
Mazhar Özkan
Tekirdag Namik Kemal University: Tekirdag Namik Kemal Universitesi

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Abstract

The synchronization of astrocytes via gap junctions (GJ) is a crucial mechanism in epileptic conditions, contributing to the synchronization of the neuronal networks. Little is known about the endogenous response of GJ in genetic absence epileptic animal models. We evaluated and quantified astrocyte GJ protein connexin 30 (Cx30) and 43 (Cx43) in the somatosensory cortex (SSCx), ventrobasal (VB), centromedian (CM), lateral geniculate (LGN) and thalamic reticular (TRN) nuclei of thalamus of genetic absence epilepsy rats from Strasbourg (GAERS), Wistar albino glaxo rats from Rijswijk (WAG/Rij) and control Wistar animals using immunohistochemistry and Western Blot.

The Cx30 and Cx43 immunopositive astrocytes in per unite area were quantified for each region of the three animal strains. Further, Cx30 and Cx43 Western Blot was applied to the tissue samples from the same regions of the three strain.

The number of Cx30 immunopositive astrocytes showed significant increase in both GAERS and WAG/Rij compared to control Wistar in all brain regions studied except LGN of WAG/Rij animals. Furthermore, Cx43 in both GAERS and WAG/Rij showed significant increase in SSCx, VB and TRN. The percentage of dual expression of Cx30 and Cx43 in the same astrocyte ranged between 17-100% for the 5 brain regions of the 3 animal strains studied. The protein expression of both Cx30 and Cx43 in the two epileptic strain showed an increase compared to Wistar control animals.

The significant increase in the astrocytic GJ proteins Cx30 and Cx43 and the differences in the dual expression of Cx30 and Cx43 in the genetically absence epileptic strains compared to control animals may suggest that astrocytic Cx's may be involved in the mechanism of absence epilepsy. Increased number of astrocytic Cx's in GAERS and WAG/Rij may represent a compensatory response of the thalamocortical circuitry to the absence seizures or may be related to the production of absence seizures.

1. Introduction

Astrocytes are not a simple supporting cell of the central nervous system; in fact play a more active role in higher neural processing than previously recognized. The knowledge related to the heterogeneity and complexity of astrocytes and its association with neurons has significantly increased in the last 20 years (Nedergaard et al. 2003; Pannasch et al. 2011; Dallérac and Rouach 2016). Astrocytes play a key role in large spectrum of complex and essential functions of the central nervous system (CNS). These include synaptic function, regulation of blood flow and fluid, ion, pH and transmitter homeostasis and development of the CNS (Barres 2008; Araque et al. 2014; Dallérac and Rouach 2016).

Astrocyte dysfunction has been association with many neurological disorders of the CNS such as; stroke and cerebrovascular disease (Carmichael 2006), CNS trauma (Sofroniew 2009), multiple sclerosis (Kuhlmann et al. 2008), autoimmune inflammatory disorders (Nishie et al. 2004), Alzheimer's disease (Nagele et al. 2004), Huntington's disease (Gil and Rego 2008), amyotrophic lateral sclerosis (Rothstein et al. 1992), psychiatric disorders (Halassa et al. 2009), brain tumors (Furnari et al. 2007), epilepsy

(Margareta Melø et al. 2007), and many other diseases. Astrocytes respond to all forms of CNS insults through a process referred to as reactive astrogliosis, which has become a pathological verification of CNS structural lesions (De Keyser et al. 2008).

The intercellular communication between astrocytes and neuronal cells is fundamental for the development and normal function of the CNS. Gap junctions (GJs) are complex structures constituted by protein channels called connexins (Cx's) (Nagy and Rash 2000). Cx's form specialized clusters of intercellular channels that allow the intercellular exchange of ions and small molecules and facilitate signaling which is important in the maintenance of brain homeostasis (Nedergaard et al. 2003; Seifert et al. 2006).

Absence epilepsy has been proposed to be associated with an imbalance between inhibitory (GABA) and excitatory (glutamate) neurotransmitters. One of the major functions of astrocytes is to enwrap pre- and post-synaptic neurons (tripartite synapse) and modulate neuronal activity by uptake and release of neurotransmitters in the extracellular fluid via GJs (Halassa et al. 2007, 2009; Araque et al. 2014).

Absence epilepsy is characterized by concomitant periodic generalized spike-wave discharges (SWD) in the electroencephalogram (EEG) with an alteration of consciousness and behavioral arrest (Tenney and Glauser 2013). The Wistar Albino Glaxo rats from Rijswijk (WAG-Rij) and the Genetic Absence Epilepsy Rat from Strasbourg (GAERS) are two well-validated genetic rat models of typical absence epilepsy that fit well with those observed in human patients with typical absence epilepsy (Vergnes et al. 1987; Danober et al. 1998).

The disruption of glutamate homeostasis modulated by astrocytic GJ may leads to synapse dysfunction and excitotoxicity and may play a role in absence epilepsy. Furthermore, the synchronization of astrocytes via GJ, modulates neuronal function which is considered to be a crucial mechanism in epileptic conditions, contributing to the synchronization of the neuronal cell networks, possibly inducing recurrent epileptiform activity (Fellin and Carmignoto 2004; Halassa et al. 2007). Our previous study showed an increase in GFAP-positive astrocyte populations and GFAP protein expression levels between Wistar and genetically epileptic strains (Çavdar et al. 2019). The present study aimed to quantify astrocyte GJ transmembrane protein Cx30 and Cx43 using immunohistochemistry and protein expression (Western blot) in the somatosensory cortex (SSCx), centromedian (CM) ventrobasal (VB), reticular (TRN) and dorsal lateral geniculate (LGN) thalamic nuclei in Wistar, GAERS and WAG/Rij animals and compare the result in the three strain.

2. Materials And Methods

In the present study total of 27 adult animals were used (n = 9 Wistar, n = 9 GAERS and n = 9 WAG/Rij) and their weights ranged from 370 to 420 gm with a mean weight of 397 gm. The institutional Animal Care and Use Committee of Koç University approved all procedures. Animals were maintained in animal

care facilities at the Koç University, on a 12:12 light/dark cycle at a constant room temperature of 72^0F, with water and Purina Rodent Chow ad libitum.

2.1. Cx30 and Cx43 immunohistochemistry

The rats were anesthetized with an intraperitoneal injection of ketamine (100 mg/kg) and xylazine (30 mg/kg). The animals were transcardially perfused with phosphate-buffered saline (PBS, 10 mM, pH 7.4) and ice-cold fixative solution (4% paraformaldehyde in 0.1 M phosphate buffer at pH 7.4) using Monostat Vera peristaltic pump (Thermo Fisher Scientific, MA, USA). Brains were removed immediately and post-fixed in the 4% paraformaldehyde overnight at 4°C. Tissues were processed into paraffin blocks and coronal sections were cut 5µ thick using microtome (Leica RM2235, Leica Biosystems, Germany). Sections were deparaffinized and antigen retrieval was performed using microwave oven. After wash 0.3% hydrogen peroxide was applied for 10 min to block endogenous peroxidase activity. After rinsing with PBS, to avoid non-specific binding, sections were incubated for one hour in 10% goat serum and 0.1% Triton X-100 in PBS at room temperature. Subsequently, the sections were incubated with Cx30 (1:100 dilution; Santa Cruz Biotechnology Inc., CA, USA) monoclonal and Cx43 (1:1000 dilution; Abcam, MA, USA) polyclonal primary antibodies diluted in 3% bovine serum albumin and 0.1% Triton X-100 in PBS at 4°C overnight. After rinsing with PBS, sections were incubated for 15 min with biotinylated goat anti-polyvalent secondary antibody. The sections were washed and incubated with streptavidin peroxidase for 10 min. After several PBS washes, tissues were exposed to 3,3-diaminobenzidine (DAB). Finally, the sections were rinsed again in PBS, counterstained with hematoxylin, dehydrated, and cover slipped with Entellan (Merck, Darmstadt, Germany). For an accurate quantification to distinguish neurons from astrocytes the sections were counterstained with Hematoxylin (Fig. 1A, B, C, D, E). Images were captured at 40X magnification under a light microscope. Quantification was performed using Image J (NIH, Bethesda, MD, USA).

2.2. Quantification of Cx30 and 43 immunopositive astrocytes

Counts were made by a single observer who was blinded to details regarding the strains (Wistar, WAG/Rij or GAERS) of the animals. The counts were made in standard 0.08mm^2 unit area using semi quantitative method. Four to six sections from each brain region for each animal were photographed at X40 magnification and counted, the averaged was expressed as the mean number of astrocytes per unite area (Table 1). The counts were made from standard brain regions. The quantifications were made from sections obtained from the following coordinates for each brain region: the SSCx (AP: 1.80 to -1.92 mm from bregma), the CM (AP: -2.04 to -3.00 from bregma), the VB (AP: -2.64 to -3.96 mm from bregma), the TRN (AP: -1.80 to -2.52 mm from bregma) and LGN (AP: -3.60 to -4.44 mm from bregma) (Paxinos and Watson 2013). One-way ANOVA (Kruskal-Wallis test) with Dunn's posttest and Mann-Whitney U-test were performed using GraphPad Prism (San Diego California USA), version 5.00 for Windows, was used for the comparison of numbers of Cx-immunopositive cells of each region. A preliminary study indicated that a

systematic random sampling of every four section through the structure of interest generated a coefficient of error < 10%.

2.3. Western Blotting

The animals were sacrificed by cervical dislocation and the brains were removed immediately and placed on the rat brain slicer. The brain regions of interest were excised and placed in a nitrogen tank and stored at -80^{0}C. As the tissue was not fixed and the TRN occupies a very narrow region in the brain we were not confident in the excision of the TRN thus, we excluded the TRN in the Western Blot part of the study.

The western blotting was done as previously described in our recent study (Çavdar et al. 2019). The tissue cellular proteins of each experimental rat was isolated for 30 minutes incubation with T-PER ®Tissue Protein Extraction Reagent (Thermo Scientific, USA), 0.1 mM PMSF, and 1X protease inhibitor cocktail (complete Protease Inhibitor Cocktail Tablets, Roche). The tissues were sonicated and centrifuged (12000 rpm, 4°C, 15 min). The protein containing supernatants from the tissues were denatured in 4xSDS sample buffer at 95°C for 5 minutes. The protein concentration of each sample was determined with BCA Protein Assay Kit (Thermo-Scientific) and equal amounts of protein were separated by 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis. The proteins electrophoretically transferred to polyvinylidene difluoride membrane by Trans-Blot® TurboTM RTA Mini PVDF Transfer Kit (Biorad) for 30 min, then the membranes were blocked with 5% non-fat for 1 h at room temperature. Next, the membranes were incubated with primary antibodies at 4°C overnight. The Connexin30 (514847, Santa Cruz Biotechnology), Connexin43 (11370, Abcam) and α-tubulin (15246, Abcam) primary antibodies were used. The membrane was washed three times with TBS-T for 15 min. The corresponding appropriate horseradish peroxidase coupled secondary antibodies (Cell Signaling, 1:10.000) were incubated for 1 h, and the membrane was washed three times with TBS-T. The proteins in the membranes were visualized by Odyssey Scanner (LiCor Biosciences) after incubating the membranes with ClarityTM Western ECL Substrate (Bio-Rad). Protein quantification was performed by densitometry analysis of Image J software (Image J, National Institutes of Health, Bethesda, MD, USA).

3. Results

The mean number of Cx30 and Cx43 immunopositive astrocytes in standard 0.08mm^2 unit area of the SSCx, VB, LGN, CM and TRN of GAERS, WAG/Rij and control Wistar animals are stated in Table 1. The number and the ratio of immunopositive Cx30 (Fig. 2) and Cx43 (Fig. 3) showed significant difference between the 5 (SSCx, VB, CM, LGN and TRN) regions of the 3 strains (see table 1). The ratio of Cx30 to Cx43 in all 5 regions of the 3 strains studied ranged between 0.7–1.3 (Table 2).

Table 1

The table shows the mean number of Cx30 and Cx43 and the ratio of immunopositive astrocytes in 0.08mm^2 unit area of the SSCx, VB, LGN, CM and TRN of GAERS, WAG/Rij and control Wistar animals.

	SSCx (SD)	VB (SD)	LGN (SD	CM (SD)	TRN (SD)
Wistar Cx30	28.6 ± 3.6	18.9 ± 1.7	21.9 ± 2.3	25.8 ± 3.5	21.0 ± 2.1
Wistar Cx43	21.8 ± 2.4	21.7 ± 1.3	29.8 ± 2.5	25.3 ± 2.5	22.1 ± 2.6
Wistar Ratio Cx30/Cx43	1.3	0.9	0.7	1.0	1.0
GAERS Cx30	48.4 ± 5.7	28.1 ± 1.9	27.6 ± 2.2	32.3 ± 4.2	24.8 ± 2.0
GAERS Cx43	49.3 ± 1.9	24.8 ± 1.7	25.7 ± 2.4	27.1 ± 3.5	26.7 ± 3.7
GAERS Ratio Cx30/Cx43	1.0	1.1	1.1	1.2	0.9
WAG/Rji Cx30	30.9 ± 2.2	25.6 ± 2.7	21.3 ± 2.4	39.1 ± 2.8	28.4 ± 3.9
WAG/Rji Cx43	45.2 ± 4.3	25.6 ± 1.9	28.8 ± 2.7	36.8 ± 4.4	30.7 ± 4.3
WAG/Rij Ratio Cx30/Cx43	0.7	1.0	0.7	1.1	0.9

Table 2

The table shows the sum of Cx30 and Cx43 immunopositive astrocytes and the percentage of dual expression in 0.08mm^2 unit area of the SSCx, VB, LGN, CM and TRN of GAERS, WAG/Rij and control Wistar animals.

	SSCx	VB	LGN	CM	TRN
Av. No of astrocyte in Wistar (data from Çavdar et al.)	32.4 ± 2.2	20.8 ± 2.1	31.4 ± 2.3	34.6 ± 2.4	21.5 ± 3.4
Av. No of Wistar Cx30 + Cx43	50.4 ± 6.0	40.6 ± 3.0	51.7 ± 4.8	51.1 ± 6.0	43.1 ± 4.7
Dual expression of Cx30 & Cx43 in Wistar (%)	55.6	95.2	64.7	47.7	100
Av. No of astrocyte in GAERS (data from Çavdar et al.)	54.8 ± 3.2	35.7 ± 4.1	41.1 ± 4.5	50.8 ± 4.8	27.9 ± 3.1
Av. No of GAERS Cx30 + Cx43	97.7 ± 7.6	52.9 ± 3.6	53.4 ± 4.6	59.4 ± 7.7	51.5 ± 5.7
Dual expression of Cx30 & Cx43 in GAERS (%)	78.3	48.2	29.7	16.9	84.6
Av. No of astrocyte in WAG/Rij (data from Çavdar et al.)	52.4 ± 4.0	33.5 ± 2.4	34.8 ± 4.1	47.9 ± 2.8	39.5 ± 3.7
Av. No of WAG/Rij Cx30 + Cx43	76.1 ± 7.5	51.7 ± 4.6	50.1 ± 5.1	75.9 ± 7.2	59.1 ± 8.2
Dual expression of Cx30 & Cx43 in WAG/Rij (%)	45.2	54.3	43.9	58.5	49.6

3.1. The quantification of Cx30 immunohistochemistry

The correlation of the mean number of Cx30 immunopositive astrocytes of GAERS in all brain regions (SSCx, $p < 0.0001$; VB, $p < 0.0001$; LGN, $p < 0.0001$, CM, $p < 0.0001$ and TRN ($p = 0.005$) showed statistically significant increase compared to control Wistar animals (Table 1, Fig. 4A). The WAG/Rij animals showed significant increase in the all (SSCx, $p < 0.0001$; VB, $p < 0.0001$; CM, $p < 0.0001$ and TRN $p = 0.0038$) regions except LGN ($p = 0.73$) compared to control Wistar animals (Table 1, Fig. 4A). The comparison of the mean number of Cx30 immunopositive astrocytes of GAERS with WAG/Rij animals showed significant increase in the SSCx ($p = 0.002$), VB ($p = 0.002$) and LGN ($p < 0.0001$) of GAERS compared to WAG/Rij animals (Table 1, Fig. 4A). However, the immunopositive astrocytes in the CM ($p < 0.0001$) and TRN ($p = 0.022$) in WAG/Rij was significantly higher than GAERS.

3.2. The quantification of Cx43 immunohistochemistry

The mean number of Cx43 immunopositive astrocytes in the SSCx ($p < 0.0001$), VB ($p = 0.0004$) and TRN ($p = 0.005$) of GAERS were significantly high compared to Wistar animals. However, LGN ($p = 0.0003$) of Wistar animals showed a significant increase in the mean number of Cx43 immunopositive astrocytes compared to GAERS animals (Table 1, Fig. 4B). The WAG/Rij animals showed significant increase in the SSCx ($p < 0.0001$) and VB ($p < 0.0001$), CM ($p < 0.0001$) and TRN ($p = 0.0003$) thalamic nuclei compared

to control Wistar animals (Table 1, Fig. 4B). The correlation of the mean number of Cx43 immunopositive astrocytes of GAERS with WAG/Rij animals showed significant increase in WAG/Rij in the LGN ($p = 0.0007$) and CM ($p < 0.0001$) TRN ($p = 0.029$) compared to GAERS animals (Table 1, Fig. 4B). However, significant decrease number of immunopositive astrocytes were observed in the SSCx ($p = 0.0015$) of WAG/Rij animals compared to GAERS.

3.3 Western Blot results

The immunohistochemistry results of the present study were correlated with Western Blot analysis. The protein expression of Cx30 was increased in the VB and LGN compared to Cx43 (Fig. 5A, B, C, D). However, Cx43 protein expression was increased compared to Cx30 in the SSCx and CM thalamic nucleus (Fig. 5A, B, C, D). Both the GAERS and WAG/Rij animals showed a significant increase in Cx30 and Cx43 protein expression in all brain regions studied compared to Wistar control animals (Fig. 5E-H). The results of the protein expression of Cx30 in the SSCx, CM, VB and LGN are highly correlated with immunocytochemical results obtained from GAERS and control Wistar animals. However, WAG/Rij animals showed correlations between SSCx and VB thalamic nuclei for both Cx30 and Cx43 (Fig. 4A, B, Fig. 5E-H).

4. Discussion

4.1. Major findings

The present study showed an increase in the number of Cx30 immunopositive astrocytes in all regions studied in GAERS and WAG/Rij compared to control Wistar animals, except LGN of WAG/Rij. Furthermore, GAERS animals showed significant increase in Cx43 immunopositive astrocytes in the SSCx, VB and TRN, whereas, WAG/Rij animals showed significant increase in the SSCx, VB, TRN and CM. The Western Blot results highly correlated with the immunohistochemistry results showing significant increase in Cx30 and Cx43 protein expression of GAERS and WAG/Rij compared to Wistar control animals in all brain regions studied.

4.2. Absence epilepsy and Cx's

The Cx's exhibit cell-specific distribution in the brain. The majority of neuronal Cx's are composed of Cx26, Cx36 and Cx40 whereas astrocytes are composed of Cx30 and Cx43. Studies have shown the expression pattern of Cx30 and 43 is heterogeneous throughout the CNS (Nagy and Rash 2000; Nagy et al. 2004; Swayne and Bennett 2016; Vicario et al. 2017). In accordance with the former studies, the present study also showed differences in the number ratio of Cx30 and Cx43 in 5 brain regions of 3 strains (Table 1). Thus, the varying number and ratio of the two Cx's studied may suggest functional specialization in GJ based on different functional requirements among CNS regions studied. Moreover, the difference observed in the 2 epileptic strains may be the cause of the SWD's of absence seizures or may represent a compensatory response.

The major electrophysiological hallmark of absence seizures are SWDs. Evidence from animal and human studies suggests that SWD are caused by a disruption of thalamic circuitry, with extensive spread to cortex through thalamo-cortical propagation in absence epilepsy. Specific regions of the cortex (forelimb, barrel field, perioral region of the somatosensory cortex) and thalamus (VB and TRN) are involved for the generation and spreading of the epileptic discharges (Nersesyan et al. 2004). The results of the present study showed an increase in the both Cx30 and Cx43 immunopositive astrocytes and Cx protein expression in the two genetically absence epileptic strains compared to control Wistar animals, in brain regions involved in the thalamo-cortical circuit (SSCx, VB and TRN). In our previous study we showed a significant increase in the GFAP-positive astrocyte populations and GFAP protein expression levels in the genetically epileptic strains (GAERS and WAG/Rij) compared to control Wistar animals (Çavdar et al. 2019). The sum of the immunopositive Cx30 and Cx43 astrocytes was higher than the number of astrocytes counted in our previous study (Table 2). This may be due to the dual expression of Cx30 and Cx43 in a single astrocyte. Nagy et al. demonstrate that the vast majority of Cx43 immunopositive were also immunopositive for Cx30 in the hypothalamus (Nagy et al. 1999). The present also shows the dual expression of two Cx's in the thalamic and the cortical astrocytes (Table 2).

Astrocytic GJ's are critical for K^+ and glutamate buffering, calcium wave propagation, and synaptic plasticity (Pannasch et al. 2011; Lapato and Tiwari-Woodruff 2018). Glutamate is the most abundant neurotransmitter in CNS and is released by excitatory neurons. The residual glutamate in the extracellular space is, potentially, neurotoxic. One of the major functions of astrocytes is to uptake most of the synaptically-released glutamate, which optimizes synaptic transmission prevents glutamate excitotoxicity (Fellin and Carmignoto 2004; Mahmoud et al. 2020). Absence epilepsy have been proposed to be associated with an imbalance in the level of □-aminobutyrate (GABA) and glutamate neurotransmitters (Panayiotopoulos 2001; Cope et al. 2009). Studies have reported drugs that increase GABAergic function aggravate absence seizures in patients with absence seizures (Panayiotopoulos 2001). In addition, it has been demonstrated that the enhancement of tonic $GABA_A$ receptor function in the thalamus of genetic absence epilepsy models is due to a malfunction of the astrocytic GABA transporter GAT-1 (Cope et al. 2009). Yamamura et al. demonstrated that ONO2506 (glial modulating agent) inhibited the spontaneous absence epileptic seizures of Cacna1atm2Nobs/tm2Nobs mice (Yamamura et al. 2013). The discrepancy observed in the number and expression of Cx30 and Cx43 GJ channels proteins in the present study may suggests an imbalance of the opposing functions of glutamate uptake and release of astrocyte in absence epileptic stains compared to control rats (Table 2).

Altered expression of Cx's have been frequently reported to be associated with various disease including mesial temporal lobe epilepsy (MTLE) (Fonseca et al. 2002; Steinhäuser et al. 2012). However, scarce data is present related to absence epilepsy. A bilateral microinjection of astrocytic GJ inhibitor carbenoxolone into TRN and nucleus ventralis posterolateralis (VPL) thalami produced a decrease in the duration and the number of SWDs in WAG/Rij rats and lh/lh mice (Gareri et al. 2005). A recent study by Vincze et al. compared the seizures of MTLE (low-[Mg2+] in vitro model) with absence epilepsy (WAG/Rij) by modulation of astrocytic GJs. Opening of GJs by trimethylamine intensifies seizures in MTLE, while

alleviates seizures in the WAG/Rij rats. In contrast, application of the GJ blocker carbenoxolone prevents the seizures in MTEL model, but aggravates seizures in absence epilepsy. They conclude that GJ openers and blockers play an opposite role on epileptiform activity in the two epilepsy model (Vincze et al. 2019). In accordance with the above results the present study showed an increase in the astrocytic Cx's in the thalamo-cortical structures (SSCx VB and TRN) in the two epileptic strains which may be related to the underlying mechanism involved in the generation and maintenance of seizures.

4.3. Comparison of GAERS and WAG/Rij

The results of the present study and former studies showed differences between GAERS and WAG/Rij. These include the age of onset and intensity of SWDs, cortical focus localization, distribution of D2-like dopamine receptors and genetic differences (Danober et al. 1998; Coenen and Van Luijtelaar 2003; Birioukova et al. 2005). The results of the present study also showed differences in the Cx30 and Cx43 protein expressions and in the number, ratio of immunopositive Cx30 and Cx43 between the two genetically absence epileptic strains (Table 1,2, Fig. A, B, 5A-H). The difference in the number, ratio and expression of Cx30 and Cx43 observed in GAERS and WAG/Rij may show the different epileptic mechanism of the two strains.

Conclusion

Astrocytes dynamically interact with neurons to regulate synaptic transmission. The enhanced excessive Cx protein expression and number of immunopositive astrocytes in the genetically absence epileptic strains especially in the SSCx, VB and TRN compared to control animals can be related to the underlying mechanism involved in the generation and maintenance of seizures of absence epilepsy. The present study may contribute and pave the way for the development of astrocyte-related therapeutic interventions for the pharmacological treatment of absence seizures.

Declarations

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Conflict of Interest Statement:

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Disclosure statement:

All authors declare that they have no actual or potential conflict of interest.

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