Comparing astrocytic gap junction of genetic absence epileptic rats with control rats: an experimental study

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Received: 19 January 2021 / Accepted: 26 May 2021 / Published online: 7 June 2021
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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 (Cx) 30 and 43 in the somatosensory cortex (SSCx), ventrobasal (VB), centromedian (CM), lateral geniculate (LGN) and thamic 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 per unit area were quantified for each region of the three animal strains. Furthermore, Cx30 and Cx43 Western Blot was applied to the tissue samples from the same regions of the three strains. 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 protein expression was increased in both Cx30 and Cx43 in the two epileptic strains compared to control Wistar animals. The significant increase in the astrocytic GJ proteins Cx30 and Cx43 and the differences in the co-expression of Cx30 and Cx43 in the genetically absence epileptic strains compared to control Wistar 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 and/or development of absence seizures.

Keywords Astrocytes · Gap junction · Absence epilepsy · Immunohistochemistry · Connexin · Protein expression

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; Pan, nasch et al. 2011; Dallérac and Rouach 2016). Astrocytes play a key role in a 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; Dallé rac and Rouach 2016).

Astrocyte dysfunction has been associated 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 (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).

The major electrophysiological hallmark of absence seizures is spike-wave discharges SWDs (Tenney and Glauser 2013). The SWD’s underlying absence seizures are generated within the cortico-thalamocortical network that involves the somatosensory cortex (SSCx), the reticular thalamic nucleus (TRN), and the ventrobasal (VB) thalamic nuclei. An increased excitatory drive from the SSCx to the TRN generates pathological bursts of TRN GABAergic neurons leading to GABA_B receptor-mediated inhibitory post-synaptic potentials in VB thalamic neurons. The resulting hyperpolarization enhances the activity of T-type voltage-sensitive calcium channels in VB thalamic neurons producing the pathological 3–4 Hz oscillations that are typical of absence seizures (Nersesyan et al. 2004). 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 engulf pre- and post-synaptic neurons (tripartite synapse) and modulate neuronal activity by uptake and release of neurotransmitters in the extracellular fluid via GJs (Holassa et al. 2007, 2009; Araque et al. 2014). The disruption of glutamate homeostasis modulated by astrocytic GJ may lead 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 (Fallin and Carmignoto 2004; Halassa et al. 2007). Our previous study showed an increase in GFAP-positive astrocyte populations and GFAP protein expression levels between control Wistar and genetically epileptic strains (Çavdar et al. 2019). The present study aimed to quantify astrocyte GJ whether changes in the transmembrane protein Cx30 and Cx43 using immunohistochemistry, immunofluorescence and protein expression (Western blot) in the somatosensory cortex (SSCx), centromedian (CM) ventrobasal (VB), reticular (TRN) and lateral geniculate (LGN) thalamic nuclei in Genetic Absence Epilepsy Rat from Strasbourg (GAERS) and Wistar Albino Glaxo rats from Rijswijk (WAG/Rij) and control Wistar animals and compare the result in the three strain.

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 g. 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 °F, with water and Purina Rodent Chow ad libitum.

Cx30 and Cx43 immunohistochemistry

Total of six animals from each strain were used for 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 Monotest 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 (cat. no. sc-514847; 1:100; Santa Cruz Biotechnology) mouse monoclonal and Cx43 (cat. no. ab11370; 1:1000; Abcam) rabbit 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, dehydrated, and coverslipped with Entellan (Merck, Darmstadt, Germany). Images were captured at 40X magnification under a light microscope. Quantification was performed using Image J (NIH, Bethesda, MD, USA).
Quantification of Cx30 and Cx43 immunopositive astrocytes

Counts were made by a single observer who was blinded to details regarding the strains (control Wistar, WAG/Rij or GAERS) of the animals. The counts were made in standard 0.08 mm² unit area using a 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 unit 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 mm from bregma), the VB (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 sections through the structure of interest generated a coefficient of error <10%.

Western blotting

Three animals from each strain were used for Western Blotting. The animals were sacrificed by decapitation and the brains were removed immediately and placed in the rat brain slicer. The brain regions of interest were excised and placed in a nitrogen tank and stored at −80°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 were isolated for 30 min 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 (12,000 rpm, 4 °C, 15 min). The protein containing supernatants from the tissues were denatured in 4x SDS sample buffer at 95°C for 5 min. 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 were 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 1 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) and normalized with α-tubulin. The western blot was analyzed according to previous study using three rats for each group (Han et al. 2021). For statistical analysis of western blot, Student’s t test was used, and data are the mean ± SD from three independent experiments. p

| Table 1 | The table shows the mean numbers and standard deviations of Cx30 and Cx43 immunopositive astrocytes in 0.08 mm² unit area of the SSCx, VB, LGN, CM, and TRN of GAERS, WAG/Rij and control Wistar animals |
|-------------|-------------|-------------|-------------|-------------|-------------|
|           | SSCx (mean ± SD) | VB (mean ± SD) | LGN (mean ± SD) | CM (mean ± SD) | TRN (mean ± 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/Rij Cx30| 30.9 ± 2.2      | 25.6 ± 2.7      | 21.3 ± 2.4      | 39.1 ± 2.8      | 28.4 ± 3.9     |
| WAG/Rij 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               |

The ratio of Cx30 to Cx43 were calculated
values < 0.05 (*), < 0.001 (**), < 0.0001 (***) were considered significant.

Double fluorescent Cx30 and Cx43 immunolabeling

Five μm thick sections were deparaffinized and antigen retrieval was performed with sodium citrate buffer (0.01 M, pH 6.0). The slices were permeabilized in 0.1% Triton X-100 in PBS for 15 min and blocked using 10% normal goat serum (Cell Signaling) with 0.1% Triton X-100 in PBS for 1 h. Subsequently, the samples were incubated with primary Cx30 (sc-514847; 1:500; Santa Cruz Biotechnology) monoclonal and Cx43 (ab11370; 1:500; Abcam) polyclonal primary antibodies overnight at 4 °C. After washing four times in PBS, slices were incubated in the secondary antibodies conjugated with Alexa Fluor 488 (A-11008; 1:500; Invitrogen) and Alexa Fluor 594 (A32742; 1:1000; Invitrogen) for 1 h at RT to facilitate fluorescent detection. After washing four times in PBS, slides were coverslipped using mounting medium with DAPI (ab104139; Abcam) and sealed with nail polish. Images were captured using confocal laser microscopy (TCS SP8 X, LEICA, Wetzlar, Germany).

Results

The immunopositive Cx30 and Cx43 astrocytes in the cortex (SSCx) and in the thalamic nuclei (VB, LGN and the CM) were quantified. All quantifications were made from standard region and layers (layers V and VI) of the SSCx. The distinct cell populations and the organization of each thalamic nuclei (VB, LGN, CM and TRN) made it easily separable from other CNS structures subsequent to Cx immunolabeling. The VB complex had a lobulated appearance imposed on it by penetrating bundles of myelinated thalamocortical fibers (Fig. 1). The LGN was apparent on the dorsolateral surface of the thalamus and contained a laminated structure (Fig. 1). The CM thalamic nuclei situate across the midline and form thin sheaths and contained large cells (Fig. 1). The TRN forms a sheath over the thalamus and this sheath is broken up by bundles of fibers of the thalamic radiations as they pass through it. The Cx30 and Cx43 immunopositive astrocytes were scattered within the thalamic nuclei (Fig. 1).

The quantification of Cx30 immunohistochemistry

The comparison of the mean number of Cx30 immunopositive astrocytes of GAERS in all brain regions (SSCx, VB, CM and TRN of GAERS, WAG/Rij and control Wistar animals only showed significant increase in the SSCx, VB, CM and TRN p < 0.0001 and TRN p = 0.005) showed statistically significant increase compared to control Wistar animals (Table 1, Fig. 2a). The WAG/Rij animals showed significant increase in all (SSCx, VB, CM and TRN p < 0.0001 and TRN p = 0.0038) regions except LGN (p = 0.073) compared to control Wistar animals (Table 1, Fig. 2a). The comparison of the mean number of Cx30 immunopositive astrocytes of GAERS with WAG/Rij animals showed significant increase in the SSCx (p < 0.001), VB (p = 0.002) and LGN (p < 0.0001) of GAERS animals, however, a significant decrease in the CM (p < 0.0001) and TRN (p = 0.022) was observed (Table 1, Fig. 2a).

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 control Wistar animals. However, LGN (p = 0.0003) of control Wistar animals showed a significant increase in the mean number of Cx43 immunopositive astrocytes compared to GAERS animals (Table 1, Fig. 2b). 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. 2b). The comparison of the mean number of Cx43 immunopositive astrocytes of GAERS with WAG/Rij animals showed a significant increase in LGN (p = 0.0007) and CM (p < 0.0001) thalamic nuclei of WAG/Rij animals, however, significant decrease was observed in the SSCx (p = 0.0015) of WAG/Rij animals compared to GAERS (Table 1, Fig. 2b).

Western blot

The immunohistochemistry results of the present study were compared with Western Blot analysis. The protein expression of Cx30 in GAERS showed significant increase in the SSCx, VB, LGN and CM compared to control Wistar animals, however, WAG/Rij animals showed significant increase in the SSCx, VB and LGN of thalamus (Fig. 3a–d). The protein expression of Cx43 in GAERS showed significant increase in the SSCx and CM of thalamus compared to control Wistar animals, however, WAG/Rij animals only showed significant increase in the SSCx and VB thalamic nucleus (Fig. 3a–d). The results
of the protein expression of Cx30 in the SSCx, CM, VB and LGN highly corresponded to immunohistochemistry results obtained from GAERS and control Wistar animals. However, the western blotting results of SSCx and VB thalamic of WAG/Rij animals coincide with immunohistochemistry results for both Cx30 and Cx43 (Fig. 3a–d).
Double fluorescent Cx30 and Cx43 immunolabeling

The double fluorescent immunolabeling showed co-localization of Cx43 staining with the Cx30 in the astrocytes in all regions studied. The number of Cx43 was apparently numerous than Cx30 in all regions studied (Fig. 4a–c).

Discussion

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 corresponded with the immunohistochemistry results showing significant increase in Cx30 and Cx43 protein expression of GAERS and WAG/Rij compared to control Wistar animals in epileptic focal areas (SSCx and VB) of the brain regions studied.

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 Cx43 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 numbers ratios of Cx30 and Cx43 in five brain regions of three strains representing fold changes of band intensities of Cx43 and Cx30 for Wistar, GAERS and WAG/Rij animals. Quantification of western blot analysis was obtained with relative densitometry and normalized with α-tubulin. Data (n = 3 blots) represent the mean (± standard deviation, SD) of three independent experiments; and is presented relative to control. *p < 0.05, **p < 0.001, ***p < 0.0001.

Fig. 3 Western Blot showing Cx43 and Cx30 protein expression levels in the SSCx, VB, LGN, and CM of GAERS, WAG/Rij and control Wistar animals. a–d Tissue lysates of WAG/Rij and control Wistar animals were separated on a 12% SDS-PAGE gradient gel and probed after transfer to PVDF membranes with mAb Cx43 and Cx30. α-Tubulin was used to assess equal loading. e–h. Histograms showing fold changes of band intensities of Cx43 and Cx30 for Wistar, GAERS and WAG/Rij animals. Quantification of western blot analysis was obtained with relative densitometry and normalized with α-tubulin. Data (n = 3 blots) represent the mean (± standard deviation, SD) of three independent experiments; and is presented relative to control. *p < 0.05, **p < 0.001, ***p < 0.0001.
(Table 1). Thus, the varying numbers and ratios of the two Cx’s studied may suggest functional specialization in GJ based on different functional requirements among CNS regions studied.

The major electrophysiological hallmark of absence seizures is SWDs. Evidence from animal and human studies suggests that SWD’s 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 in the generation and spreading of the epileptic discharges (Nersesyan et al. 2004).

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 has been proposed to be associated with an imbalance in the level of α-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\textsubscript{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 ONO-2506 (glial modulating agent) inhibited the spontaneous absence epileptic seizures of Cacna1atm2Nobs/tm2Nobs mice (2013). The results of the present study showed an increase in both Cx30 and Cx43 immunopositive astrocytes and Cx protein expression in the epileptic 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). The increase in the Cx’s in the epileptic strains may be the “cause” of the SWD’s of absence seizures or may represent a “compensatory” response. The increased astrocyte Cx’s of the epileptic strains may result from excessive synchronization and excitability of the neurons observed in the epileptic focal areas of the brain (SSCx, TRN, and VB). The increase can be a compensatory response to absence epilepsy. Moreover, the hyperexcitation of the neurons in the epileptic focal areas of the brain (SSCx, TRN, and VB) can be related to the excess release of glutamate via the increased number of Cx’s observed in the epileptic strains. Thus, the increase in the Cx’s may take part in the underlying cause of absence epilepsy mechanisms. Furthermore, the increased Cx’s may increase glutamate released by hemichannels and activate extrasynaptic glutamate receptors, eventually increasing the overall excitation level of neurons observed absence epileptic seizures. The increase in the number of Cx’s in the
absence epileptic focal area may have a seizure-promoting role. The Cx30 and Cx43 may have a part in mediating intercellular trafficking of metabolites from the extracellular space. The involvement of GJ channels in the intercellular spread of Ca\textsuperscript{2+} waves would favor a proconvulsive role of the syncytium through neuronal synchronization and the spread of ictal activity. Thus, the increase in Cx’s could be both involved in or a result of the mechanism of epileptogenicity.

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 with 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). The presence of double fluorescent immunolabeling showed colocalization of Cx43 staining with the Cx30 in all regions studied, this explains the higher number of Cx30 and Cx43 compare to the number of astrocytes. Studies reported that the vast majority of Cx43 immunopositive were also immunopositive for Cx30 in the hypothalamus (White and Bruzzone 1996; Nagy et al. 1999, 2001; Wan et al. 2012). The study also shows the presence of co-expression of two Cx’s in the thalamic and the cortical astrocytes (Table 2).

Altered expression of Cx’s has 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 are presented 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-[Mg\textsuperscript{2+}] 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 MTLE 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 models (Vincze et al. 2019). In accordance with the above results, the present study showed an increase in the astrocytic Cx’s in the thalamocortical 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.

**Comparison of GAERS and WAG/Rij**

The results of the present study and other 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 (Danőber 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 (Tables 1 and 2). The difference in the number, ratio and expression of Cx30 and Cx43 observed in GAERS and WAG/Rij may suggest 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

### Table 2

The table shows the sum of Cx30 and Cx43 immunopositive astrocytes and the percentage of co-expression in 0.08 mm\textsuperscript{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. (2019)) | 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 |
| Co-expression of Cx30 and Cx43 in Wistar (%) | 55.6 | 95.2 | 64.7 | 47.7 | 100 |
| Av. no. of astrocyte in GAERS (data from Çavdar et al. (2019)) | 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 |
| Co-expression of Cx30 and 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. (2019)) | 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 |
| Co-expression of Cx30 and Cx43 in WAG/Rij (%) | 45.2 | 54.3 | 43.9 | 58.5 | 49.6 |
development of astrocyte-related therapeutic interventions for the pharmacological treatment of absence seizures.

Acknowledgements The authors would like to thank Koç University Research Center for Translational Medicine (KUTTAM) for using the facilities.

Declarations

Conflict of interest This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. All authors declare that they have no actual or potential conflict of interest.

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