Article

Extracellular S100β disrupts Bergman glia morphology and synaptic transmission in cerebellar Purkinje cells

Olga S. Belozor[1], Dariya A. Yakovleva[2], Ilya V. Potapenko[3], Andrey N. Shuvaev[2], Marina V. Smolnikova[3,4], Alex Vasilev[5], Elena A. Pozhilenkova[1,3], Anton N. Shuvaev[3]*

1 Krasnoyarsk State Medical University named after Prof. V.F. Voino-Yasenetsky, 660022, Russia, Krasnoyarsk, Partizan Zheleznyak st. 1, Department of biological chemistry, medical pharmaceutical and toxicological chemistry.
2 Siberian Federal University, 660041, Russia, Krasnoyarsk, Svobodny pr., 79, Institute of fundamental biology and biotechnology.
3 Krasnoyarsk State Medical University named after Prof. V.F. Voino-Yasenetsky, 660022, Russia, Krasnoyarsk, Partizan Zheleznyak st. 1. Research Institute of molecular medicine and pathobiochemistry.
4 Federal Research Center "Krasnoyarsk Science Center" of the Siberian Branch of the Russian Academy of Sciences, Scientific Research Institute of medical problems of the North, Partizana Zheleznyaka street, 3G, Krasnoyarsk, Russia, 660022
5 Institute of Living Systems, Immanuel Kant Baltic Federal University, Universitetskaya str, 2, Kaliningrad, Russia, 236041*
Correspondence: shuvaevanton2016@gmail.com; Tel.: +7 (391) 228 07 69

Received: date; Accepted: date; Published: date

Abstract: Astrogliosis is a pathological process which affects the density, morphology and function of astrocytes. It is a common feature of brain trauma, autoimmune diseases and neurodegeneration including spinocerebellar ataxia type 1 (SCA1), a poorly understood neurodegenerative disease. S100β is a Ca^{2+} binding protein. In SCA1, excessive excretion of S100β by reactive astrocytes and its uptake by Purkinje cells has been demonstrated previously. Under pathological conditions, excessive extracellular concentration of S100β stimulates production of proinflammatory cytokines and induces apoptosis. We modeled astrogliosis by S100β injections into cerebellar cortex in mice. Injections of S100β led to significant changes in Bergmann glia cortical organization and affected their processes. S100β also changed morphology of the Purkinje cells (PCs), causing a significant reduction of the dendritic length. Moreover, the short-term synaptic plasticity and depolarization-induced suppression of synaptic transmission, were disrupted after S100β injections. We speculate that these effects are due to Ca^{2+}-chelating properties of S100β protein. In summary, exogenous S100β induced astrogliosis in cerebellum could lead to neuronal dysfunction which resembles a natural neurodegenerative

process. We suggest that astrocytes are playing an essential role in SCA1pathology and astrocytic S100β is an important contributor to this process.

Keywords: astrocytes; S100β; Purkinje cells; short term plasticity; Ca^{2+} signaling

1. Introduction

Spinocerebellar ataxia type 1 (SCA1) is a progressive neurodegenerative hereditary disorder which affects mainly the cerebellum and brainstem. It is caused by a dynamic expansion of CAG repeats in the N-terminal coding region of *gene*ATXN1 gene on chromosome 6p23 [1, 2]. In general population, prevalence of SCA1 is only 1-2 per 100 000, but in specific ethnic groups this ratio varies [3]. SCA1 is characterized by progressive cerebellar dysfunction, dysarthria and worsening of bulbar functions. Pathological changes include neuronal loss in the cerebellum, brainstem, and degeneration of spinocerebellar tracts [4, 5]. Higher cortical functions may also be affected with symptoms including memory loss, verbal and nonverbal intellectual deficits [6]. CAG repeats encode amino acid glutamine; therefore their expansion leads to synthesis of Ataxin 1 with excessive polyglutamine tract. This affects protein folding and leads to precipitation as intracellular aggregates [7, 8]. The aggregates also contain inclusions of ubiquitin, proteasome components and chaperons [9]. In SCA1 mouse model aggregation occurs later than the first pathological sights appear and does not correlate with the disease severity [10]. Accumulation of the mutant protein leads to selective neurodegeneration in certain regions of the brain and reactive astrocytosis. Astrogliosis in SCA1 is tightly correlated with the onset and severity of disease and is not a consequence of neuronal death. Cvetanovic et al. (2015) described the astrocytic and microglial reaction in SCA1, using *non-cell selective SCA1 knock-in (*$Sca1^{154Q/2Q}$*) and PC selective B05* $SCA1^{82Q/2Q}$ mouse models. They showed that astrocytes

and microglia are activated at early stages of SCA1. Expression of *protein* Ataxin 1 in microglia and astrocytes was not essential for the activation of glia but expression in Purkinje cells was sufficient for it [11, 12].

In cerebellum, reactive astrogliosis of Bergmann glia may disrupt the spatial distribution of EAAT1 (Excitatory Amino Acids Transporter 1 or Glutamate Aspartate Transporter (GLAST)). This could result in an increase in extracellular glutamate concentrations and toxicity via NMDA-receptors [12, 13]. In addition, activated astrocytes and microglia are able to release various proinflammatory molecules. Some of them, such as Tumor Necrosis Factor α (TNFα), interleukin-6 (IL6) and monocyte chemoattractant protein-1 (MCP-1) have been implicated in neurodegeneration and negatively affect the function and survival of neurons [14–19]. S100β protein is one of the S100 Ca^{2+}-binding proteins of the S100 group which includes nearly 20 members [20]. S100 proteins form homo- and heterodimers and are able to chelate not only Ca^{2+}, but also Zn^{2+} and Cu^{2+} [21]. Binding of the ions changes the confirmation of S100 and alters their affinity to different ligands (more than 90 potential targets known currently) [20–23].

Here we model astrogliosis in cerebellum, such as seen in SCA1, by the intracortical injections of S100β. We demonstrate a significant negative impact of this astrocytic protein to the PCs morphology and synaptic transmission in the parallel fibre-Purkinje cell (PF-PC) synapse. Moreover, we demonstrate that short term synaptic plasticity, the depolarization-induced suppression of excitation (DSE) is disrupted by S100β. We speculate that these effects could be attributed to the Ca^{2+}-chelating properties of S100β.

2. Materials and Methods

All procedures for the care and treatment of animals were carried out according to the Krasnoyarsk State Medical University and Russian public standard (33215-2014) regulations and approved by the local ethical committee. Every effort was made to minimize animal suffering and to reduce the number of animals used in this study. 12 weeks old

CD-1 IGS WT mice (Charles River Laboratories) were used in this study. 4 weeks old non-cell selective SCA1$^{154Q/2Q}$knock-inn(SCA1 KI) mice with C57BL/6J background used in this work [24]. Experiments with SCA1 KI mice were generated in Gunma University (Japan) in laboratory of Neurophysiology and Neuronal Repairled by Professor H. Hirai. These were mice kindly provided by Dr. Hidehiro Mizusawa (Tokyo Medical and Dental University). Animals were kept on a 12-h light/dark cycle with free access to food and water.

2.1. Drugs and reagents

All reagents for electrophysiological experiments were from Sigma Aldrich.

Recombinant mouse S100 Calcium Binding Protein B (S100β) (Cat. No. APA567Mu01) was obtained from Cloud-Clone Corp. Concentrated stock solution of S100β was initially prepared and diluted in physiological saline (PBS) to a final concentration before use. Fluorocitrate (FC) was used as barium salt (Cat. F9634, Sigma Aldrich) as described previously (Paulsen et al., 1987). (3S)-3-[[3-[[4-(Trifluoromethyl)benzoyl]amino]phenyl]methoxy]-L-aspartic acid (TFB-TBOA) (Cat. No. 2532) was obtained from Tocris. Concentrated stock solutions of FCand TBOA were initially prepared and diluted in artificial spinocerebellar fluid (ACSF) to their final concentrations before use.

2.2. S100β injections

12-week-old (P90-P100) WT CD1 mice were anaesthetized by intraperitoneal injection of chloral hydrate solution (400mg/kg of body weight). 2,5µl of 50µM S100β in phosphate buffered saline (PBS) or PBS was stereotaxically injected into the cortex of cerebellar vermis (lobule VI) using 10µl Hammilton syringe. To reach the injection point in the vermis we used the coordinates relative to bregma: AP: -2,5 mm, ML: 0 mm, DV: 2mm.Mice were used 24 hours after the injection.

2.3. Electrophysiology

Cerebellar slices (250 µm thick) were prepared, and whole-cell recordings were conducted as described previously [25]. Briefly, mice were deeply anesthetized by intraperitoneal injection of chloral hydrate (400mg/kg of body weight) and killed by decapitation. The brain was quickly dissected and placed for one minute in an ice-cold Ringer's solution containing: 234 *mM* sucrose, 26 *mM* NaHCO3, 2.5 *mM* KCl, 1.25 *mM* NaH2PO4, 11 *mM* glucose, 10 *mM* MgSO4, and 0.5 *mM* CaCl2 0.5; pH 7.4, continuously oxygenated with 95% O2 and 5% CO2. Parasagittal slices of cerebellar vermis were made using a microslicer (Thermo Scientific; Microtom CU65). The slices were maintained in an extracellular solution containing: 125 *mM* NaCl, 2.5 *mM* KCl, 2 *mM* CaCl2, 1 *mM* MgCl2, 1.25 *mM* NaH2PO4, 26 *mM* NaHCO3, 10 *mM* D-glucose, and 0.05-0.1 *mM* picrotoxin bubbled by 95% O2 /5% CO2 gas mix at room temperature for 1h before starting the electrophysiological experiments. For current clamp whole-cell recordings from Purkinje cells (PCs) we used K-gluconate-based intracellular solution containing: 130 *mM* K-gluconate, 4 *mM* KCl, 20 *mM* HEPES, 1 *mM* MgCl2, 4 *mM* MgATP, 1 *mM* NaGTP, 0.4 *mM* EGTA (pH 7.3 adjusted with KOH). For voltage clamp whole-cell recordings from Purkinje cells (PCs) we used intracellular solution containing: 140 *mM* Cs-gluconate, 8 *mM* KCl, 10 *mM* HEPES, 1 *mM* MgCl2, 2 *mM* MgATP, 0.4 *mM* NaGTP, 0.4 *mM* EGTA (pH 7.3 adjusted with CsOH). Passive electrical properties of the PCs were estimated using averaged traces of ~10 current responses to hyperpolarising voltage pulses (from -70 to -80 mV, 200 ms duration). Fast capacitance component was automatically compensated; signal was sampled at 50 kHz and low-pass filtered at 10 kHz. No correction was made for liquid junction potentials. Analysis of electrophysiological data was performed using pClamp10 (Molecular Devices), Pachmaster software (HEKA), and Clampfit 10.5 (Axon instruments).

PCs were voltage-clamped at -70 mV to record PF EPSCs. Selective stimulation of PFs was confirmed by paired-pulse facilitation of EPSC amplitudes (at a 50-ms interstimulus interval).

To examine depolarization induced suppression of excitation (DSE), PF EPSCs were recorded every 3 s. After monitoring basal PF EPSCs

for 1 min, a single depolarizing pulse (5s from -70 to 0 mV) was applied to the recorded PC. This opens the voltage gated Ca^{2+} channels (VGCC) and releases endocannabinoids which presynaptically decrease glutamate release and suppress amplitude of PF EPSC [26]. Amplitudes of subsequent PF EPSCs were normalized to the mean value of 12 responses evoked before the induction of DSE.

2.4. Immunohistochemistry

For immunohistochemistry (IHC), anesthetized mice were perfused transcardially with 4% paraformaldehyde in 0.1 M phosphate buffer. The brain was postfixed in the same fixative overnight. The cerebellar vermis was cut into 50-µm sagittal sections. The sections were treated with rabbit monoclonal anti-calbindin D-28 k (1:500, Cloud Clone Corp., China), chicken polyclonal anti-GFAP antibodies (1:1000, Abcam, UK), rabbit polyclonal anti-S100β (1:1000, Abcam, UK). Secondary antibodies were Alexa Fluor 514-conjugated donkey anti-rabbit IgG (1:1,000, Life Technologies), Alexa Fluor 647-conjugated donkey anti-chicken IgG (1:1,000, Life Technologies) and Alexa Fluor 488-conjugated donkey anti-rabbit IgG (1:1,000, Life Technologies). Antibodies were dissolved in PBS solution containing 2% (v/v) normal donkey serum, 0.1% (v/v) Triton X-100, and 0.05% NaN3.

2.5 Confocal microscopy and morphometric analysis

Fluorescent images were obtained using confocal microscope *and original software of* Olympus, Fluoview, FV10i (Japan). Images were recorded as Z-stacks of 0.25 µm thickness with *x10 lense,* numerical aperture of 1.0, *zoom x6, at 1024x1024 resolution.* In all groups the cerebellar lobes 6 and 7 of the vermis cerebellum were used for comparison (Fig. 1). For double labeling, images from the same confocal plane were taken. Alexa Fluor 647 signal (blue) was artificially changed to red color to show S100β/GFAP colocolization in merge microphotographs (yellow) (Fig. 1 and 2).

Thickness and number of Bergmann glia processes were measured on confocal images of sagittal cerebellar slices. The number of radial glial processes for 100µm of in the molecular layer (Sup. Fig. 1A) were counted. The same 100µm line intensity profile was used to obtain the distribution of GFAP fluorescence, using the original software of the Olympus confocal microscope. Each glial process was shown as peak of GFAP/Alexa 647 fluorescence intensity. We counted the averaged thickness of these processes in each image. To avoid false positive enhancement of processes thickness we used cut-off threshold for recognition of the GFAP signal set to 10% of the maximal fluorescence intensity. To count the number of Bergmann glia cells, we measured anti-S100β positive circle- and oval-shaped signals in Purkinje cell layer. To avoid over- or underestimation, the glial cell numbers were calculated by ceiling the ratio $n=\lceil \frac{d}{d'} \rceil$, where n is the cell number, d is the length of S100β positive signal in µm and $d'=15\,\mu m$ which we took for characteristic diameter of an astrocyte. The approximate length of the dendrites of the Purkinje cells was estimated from overall thickness of the molecular layer (*Sup. Fig. 1B*), visualized using anti-calbindin/Alexa 488 staining.

2.6. Sholl analysis of Bergmann glia cells

Quantitative morphological analysis was performed in the three-dimensional (3D) mode. Using a confocal laser scanning microscope (Olympus, Fluoview, FV10i) anti-GFAP-labeled Bergmann glia was scanned in Z-stacks (80–150 consecutive focal planes at 0.25 µm interval). For Scholl analysis Z-stacks mages of soma and processes of Bergmann glia were traced on focal planes using ImageJ software. We used the Sholl method of concentric circles using an ImageJ regime (set of nested concentric spheres is centered on the cell body, and the spheres increase in size by 10µm radius) [27]. The results of Sholl analysis showed length of processes and the number of intersections per 10 µm.

2.7. Statistical and mathematical analysis

Pooled data are expressed as the mean ± SEM. Statistical analyses of differences between the groups were performed using the unpaired t-test and Mann–Whitney U test. The influence of FC and TBOA on the EPSC recovery was estimated with one-way ANOVA test. Differences were considered significant at $p<0.05$.

We estimated the dendritic and somatic capacitance by optimization of the two-term exponential series to the current response curve to the voltage step $V_{step}=10$ mV [28]:

$$I_{clamp}(t)=V_{step}\left(1/R_{ss}-A_d\tau_d e^{-\frac{t}{\tau_d}}-A_s\tau_s e^{-\frac{t}{\tau_s}}\right).$$

$$I_{clamp}(t)=V_{step}\left(\frac{1}{R_{ss}}-A_d\tau_d e^{-t/\tau_d}-A_s\tau_s e^{-t/\tau_s}\right)$$

to find the time constants τ_i. Here $R_{ss}=4$ MΩ is the input resistance and A_i are the free parameters. Indices d and s stand for dendritic and somatic components respectively. The resulting capacitance was then calculated as $C_i=\tau_i/R_m \ (i=d,s)$ $C_i=\frac{\tau_i}{R_m}(i=d,s)$. R_m is the membrane resistance. Optimization was made in ClampFit 10.7 software.

DSE was analyzed using dual exponential waveform equation (1).

$$DSE(t)=100+A\left(e^{\frac{-t}{\tau_1}}-e^{\frac{-t}{\tau_2}}\right)\left\{A=\frac{100a\tau_1\tau_2}{\tau_1-\tau_2}\right\} \quad (1)$$

This curve is convenient for the prediction of the conduction changes in synapses [29]. It contains the parameters for both decay and recovery of EPSC separately during the DSE protocol. This model

was fitted to the experimental data by the Nelder-Mead minimization of the sum of squared residuals to find “A” – the maximum EPSC decrease in percent of initial level, “τ_1“and “τ_2“ – the half-times for the EPSC to reach the minimum and to recover to the initial 100%, respectively. The parametric bootstrap technique was used to obtain the 95% confidence intervals for the parameters A, τ_1, τ_2. This analysis was performed using Python 3 package.

3. Results

3.1. Exogenous S100β alters morphology of Bergmann glia

2.5µl of 50µM S100β were injected in the cerebellar cortex to induce astrogliosis. 24 hours later widespread distribution of S100β and increased GFAP expression in cerebellar cortex were evident in lobules IV-VII (Fig. 1C compare to Fig. 1A, note that in the normal brain immunofluorescent GFAP was poorly visible). In microphotographs of S100β injected areas prominent GFAP positive striation was evident (contrast to PBS injected areas, Fig. 1B and D). To analyze the morphology of Bergmann glia we examined the number and thickness of anti-GFAP-positive glial processes in the central part of molecular layer per 100µm using line profile function (Sup. Fig. 1A and Fig. 2A and B). The average cross-section of Bergmann glia processes in S100β injected mice was increased to 3.6±0.1 µm, (364 processes from 17 areas of 3 mice) vs 2.8±0.1 µm, (358 processes from 11 areas of 3 mice) in PBS injected mice, $p = 1.25x10^{-13}$, unpaired t-test (Fig. 2C). The number of processes per 100µm longitudinal length of molecular layer in S100β injected animals was significantly decreased compared to PBS injected animals (21.4±2.0 vs 32.6±3.3, p = 0.013, t-test; Fig. 2D).The density of Bergmann glia processes was also decreased in S100β injected areas. We also measured the fraction of “GFAP-negative” space in the central part of molecular layer per 100µm using the same line profile function. In S100β injected areas (17 areas of 3 mice) it was increased to 24.0±4.5% compared with 8.1±1.6% in PBS-injected areas (11 areas of 3 mice, p = 0.015; unpaired t-test; Fig. 2E). Low Bergmann glia processes densityis mainly due to the loss of some of these cells. Indeed, the number of anti-S100β-labeled cell bodies per 100µm longitudinal length of Purkinje cell layer in S100β injected animals was significantly decreased in comparison to PBS injected animals (9.1±0.4 vs 10.9±0.5, p = 0.007, unpaired t-test; Fig. 2F).

Next, we studied single astrocyte morphology using Sholl analysis (Sholl, 1953). On images of digitally traced Bergmann glia processes (Fig. 3A) we analyzed the maximum number of these processes per cell. This number was not changed in S100β injected areas (4.9±1.2, n=12 from 3 animals) in comparison to PBS injected areas (4.8±1.7, n=10 from 3 animals, p=0.648; Mann–Whitney U test; Fig. 3B). Sholl analysis revealed an increase in the density of proximal processes in Bergmann glia after S100β injections. Within 10μm from soma in S100β injected areas (12 areas of 3 mice) Bergmal glia had 3.3±0.3 processes, while in PBS injected areas was 1.8±0.2 (p = 0.0002; 11 areas of 3 mice, Mann–Whitney U test; Fig. 3C.)

These data show that excessive extracellular S100β protein in the cerebellar cortex leads to significant changes in Bergmann glia morphology.

3.2. Extracellular S100β alters morphology of Purkinje cells

As shown above, S100β affects glia and it is well-known that disturbances in glia may lead to neuronal degeneration [30–32]. In addition, S100β could have a direct effect on Purkinje cells. We examined the effect of S100β on morphology of these neurons using IHC and their physiological state using patch clamp. To estimate the approximate dendritic length of PCs cells were visualized by anti-calbindin staining (Fig. 4A) and measured the thickness of the molecular layer (Sup. Fig. 1B). S100β injections reduced it to 120.0±5.8 μm (n=12 areas from 3 mice) compared to 150.7±6.3 μm, n = 14 areas from 3 mice in PBS injected mice (p=0,002, t-test; Fig. 4B).

Using patch clamp we estimated capacitance of dendrites and soma after subtraction of slow capacitance component from the total capacitance of PCs (see materials and methods). Slow component reflects predominantly the size of neuronal dendrites. We found a significant difference between the two groups. The capacitances of PCs dendrites in S100β injected mice were 359.4±37.5pF (n=33 cells from 8 mice) and 513.5±27.1pF (n = 52 cells from 10 mice) in PBS injected

group. (p=0,002, t-test; Fig. 3B). The capacitances of PCs soma in S100β injected mice were 34.6±4.4pF (the same cells) and 61.7±5.6pF (the same cells) in PBS injected group. (p=0,0003, t-test; Fig. 3C).

These data indicate that excessive extracellular S100β affects PCs morphology, leading to the collapse of the soma and dendrites.

3.3. Extracellular S100β alters synaptic transmission in PFs and PCs

Astrocytes control removal of glutamate from the presynaptic space [33–36]. Moreover, astrocytic secretion of S100β protein into the intercellular space leads to endocytosis of this protein by neurons and evokes various effects such as chelation of cytoplasmic Ca^{2+} [20]. For this reason, we tested whether elevated extracellular S100β affects synaptic transmission in PF-PCs synapses. S100β did not change the PF-EPSCs amplitude (Sup. Fig. 2A).We suspected that S100β will affect processes highly dependent on Ca^{2+} release, such as presynaptic glutamate secretion. However, we did not see significant differences between PPF ratio in PF-PC synapses of S100β and PBS injected mice. The PPF ratio in S100β injected mice was 1.8±0.4, n=35 cells from 8 mice vs 1.9±0.1, n =39 cells from 9 mice in PBS injected mice (p=0.722, t-test; Fig. 5A). However, S100β dramatically affected the kinetics of PF-EPSCs. While there was no statistically significant difference in the PF-EPSCs amplitude in mice injected with S100β and PBS (Sup. Fig. 2A), the rise time of PF-EPSC in S100β injected mice was prolonged to 2.7±0.1ms (n=35 cells from 8 mice), compared to 2.3±0.1ms (n = 36 cells from 9 mice) in PBS injected mice (p=0.028, t-test; Fig. 5B).

To control for the potential effect of surgery per se, we analyzed this parameter in sliced from naïve mice. No difference was found between naïve and **PBS** injected groups (Sup. Fig. 3).

In addition the decay time of PF-EPSC in S100β injected mice was 17.1±1.5ms (n=30 cells from 8 mice), while it increased to 21.6±1.5ms (n = 37 cells from 9 mice) in PBS injected mice (p=0.04, t-test; Fig. 5C).

These results demonstrate that the excessive extracellular accumulation S100β protein mainly affects kinetics of PF-EPSC, which most likely reflects changes glutamate removal from the synaptic cleft.

3.4. Similarities in changes in synaptic transmission in S100β-injected mice and Ataxin1 mutant animals.

To look for similarities between SCA1 and consequences of S100β injections we used KI mice with non-cell selective expression of mutant Ataxin 1 [24]. Mice were used at 3 weeks of age which corresponds to the early stage of neurodegenerative process. There was no statistically significant difference in the PF-EPSCs amplitude recorded in the PCs of SCA1 KI and WT mice (Sup. Fig. 2B). The PPF ratio in SCA1 KI mice was 2.1±0.1, n=10 cells from 3 mice and 1.8±0.1, n = 8 cells from 3 mice in WT mice (p=0.012, unpaired t-test; Fig. 6A). PCs from SCA1 KI mice have altered kinetics of PF-EPSCs. The average rise time of PF-EPSC in SCA1 KI mice significantly increase to 3.0±0.2ms (n=10 cells from 3 mice), compared to WT mice (2.3±0.2ms; n = 8 cells from 3 mice; p=0.038, unpaired t-test; Fig. 6B). The differences in decay time of PF-EPSC between SCA1 KI mice and WT were not significant (18.4±2.1ms, n=10 cells from 3 mice vs. 15.9±3.3ms, n = 8 cells from 3 mice; p=0.379, unpaired t-test; Fig. 6C).

3.5. Extracellular S100β alters endocannabinoid-dependent short term plasticity in PF-PC synapses

As mentioned above, we expected that S100β could affect processes which are known to depend on the cytosolic concentration of Ca^{2+}. One of such processes is DSE, which is evoked by the membrane depolarization. Depolarization leads to opening of voltage-gated calcium channels (VGCC) and an increase in the intracellular Ca^{2+}. Ca^{2+} triggers endocannabinoid release from the postsynaptic cell with consecutive activation of CB1 receptors on the presynaptic terminal, leading to a reduction in release of glutamate. We examined dynamic of PF-EPSC amplitude after 5 sec of depolarization from -70 to 0 mV. The stimulus intensity was adjusted to reach EPSC amplitude of

approximately 150 pA before DSE induction. In control mice DSE protocol reduced EPSC by 67,3±3,5% (n = 11 cells from 4 mice) which was similar to that in mice pre-injected with S100β (69,7±4,7%, n = 12 cells from 4 mice, p = 0,975, unpaired t-test; Fig. 7A). However the recovery of the PCs amplitude was significantly faster in S100β injected mice. 50 sec after the challenge amplitude returned to 93.6±2.8% of control, compared to 83.9±2.7% in PBS injected mice (p = 0.03, unpaired t-test; Fig. 7A and B).

The double waveform model fit (1) confirmed slowing of the recovery kinetics by S100β. The amplitude reduction in the DSE protocol was not significantly different in S100β injected mice compared to PBS group: 45.5 (30.6, 63.6)% vs 38.1 (31.8, 44.6)% respectively. Using the fitting protocol we calculate that the half-time for the recovery of the parameter ($\tau 2$) is significantly smaller in S100β injected mice with 29.7 (21.0, 47.2) sec in comparison with 64.5 (52.0, 85.1) sec in PBS group, $p<0.05$.

These results demonstrate that excessive extracellular S100β protein negatively affects DSE.

3.6. Effects of FC on PF-PC transmission and endocannabinoid short term plasticity in PF-PC synapses

FC inhibits astrocytic metabolism and deprives these cells of energy, this leading to an array of repercussions which ultimately undermine functions of these cells [37]. Application of FC led to a strong depression of PF-PC excitatory transmission, irrespective of whether the tissue was exposed to S100β or not (Fig 8A and B). After 10 min application the amplitude of PF-EPSC in S100β injected mice decreased up to57.7±9.0% of control (n=7 cells from 4 mice, p=0.006, paired t-test) and in PBS injected mice up to 64.4±9.9% of control (n = 7 cells from 4 mice, p=0.014, paired t-test) (Fig. 8A and B). 10 min after FC application averaged PF-EPSCs in S100β and PBS injected groups were not different (unpaired t-test, p=0.65). The rise time of PF-EPSC in PBS and S100β injected mice increased after FC treatment. In PBS group it increased from 2.3±0.2ms to 2.7±0.2ms (n = 12 cells from 4 mice,

p=0.003, paired t-test; Fig. 8C) while in S100β group it increased from 2.4±0.3ms to 3.5±0.6ms (n = 10 cells from 3 mice, p=0.003, paired t-test; Fig. 8C).

It was shown previously that astrocytes also contain CB1 receptors and could modulate the synaptic plasticity [38]. CB1 receptors in astrocytes are coupled to $G_{q/11}$-proteins and trigger PLC activation [39] and release such gliotransmitters as glutamate, ATP or d-serine [40]. This phenomenon is SNARE-dependent and highly sensitive to ATP concentration which should be reduced by FC [41, 42]. However FC did not affect expression of DSE. After FC application in PBS injected mice DSE protocol reduced EPSC by 56,8±8,7% (n = 9 cells from 4 mice) which was not statistically differ from what registered without FC in S100β-injected mice (Fig. 8E) (p=0.317, unpaired t-test). However, this comparison is compromised by the direct impact of FC on PF-induced EPSCs and should be interpreted with care.

The double waveform model fit the changes of PF-EPSC amplitudes after depolarization pulse and DSE initiation. Maximum amplitudes reduction was 51.92 (41.67, 81.41)% and 46.23 (33.28, 75.49)% for PBS and S100β respectively.

However the recovery of the PCs amplitude was significantly slower in FC treated PCs in slices from PBS-treated mice (Fig. 8F). 50 sec after the challenge, it returned to 67.5±5.4% of control, compared to untreated slices where it recovered to 83.9±2.7%, p = 0.025, unpaired t-test; Fig. 8D-F. However, FC had hardly any effect on DSE protocol in S100β injected mice where recovery of the PCs amplitude was 81.4±7.6% of PF-EPSC amplitude at 50 sec post challenge in FC vs 93.6±2.8% without FC; p = 0.181, unpaired t-test (*Fig. 8D-F*).

Double waveform model fit also did not reveal significant changes after the FC treatment.

3.7. Slowdown of glutamate uptake in Bergmann glia by TBOA leads to alteration of PF-EPSC kinetic but does not change endocannabinoid short term plasticity in PF-PC synapses

90% of all glutamate uptakes in PF-PC synapses is due to excitatory amino acid transporters EAAT1 and EAAT2, located on the membranes of astrocytes [43, 44]. We suspected that shortening of PF-EPSC decay time after S100β injection was due to facilitation of glutamate reuptake through EAATs (Fig. 5C). 500μM TBOA significantly increased decay time of PF-EPSC in PBS and S100β injected mice. In PBS injected mice decay time changed from 30.0±4.4to 39.2±6.3msec (n=16 cells from 4 mice, p=0.039 paired t-test, Fig. 9A). Note that before TBOA application decay constants were different between S100β and PBS injected groups (p=0.044, one way ANOVA) while after TBOA they reached approximately same values (p=0.64, one way ANOVA, Fig. 9A). In S100β injected mice increase was even more dramatic, from 19.2±2.7ms to 34.6±7.1ms (n=10 cells from 3 mice, p=0.031 paired t-test). Application of nonselective EAAT blocker DL-TBOA 500μM did not significantly change PF-EPSC amplitudes and rise time in cerebellum of both PBS and S100β injected mice (data not shown). TBOA did not affect PPF ratio of PF-EPSC in PBS injected mice (1.86±0.1 vs 1.84±0.1; n=16 cells from 4 mice, p=0.673 paired t-test), while in S100β injected mice it resulted in a slight but significant PPF ratio reduction from 1.9±0.2 to 1.76±0.1 (n=10 cells from 3 mice, p=0.049 paired t-test, Fig. 9B).

We examined endocannabinoid short term plasticity in PF-PC synapses after treatment of cerebellar slices with 500μM TBOA. In PBS injected mice DSE protocol was not affected by TBOA. EPSC were reduced by 59,2±5,4%;n = 15 cells from 4 mice) which was not statistically differ from that before application (56.8±5.7%, p = 0,524, paired t-test). Also, the recovery of the PCs amplitude was not affected by TBOA (Fig. 9C and D). Neither did TBOA affect the outcome of DSE protocol in S100β injected mice (74,8±9,6% reduction in TBOA, n = 11 cells from 4 mice vs 73.6±8.8% without TBOA, p = 0,789, paired t-test). Also the recovery of the PCs amplitude was unaffected by TBOA. The double waveform model yielded in the PF-EPSC amplitudes reduction during DSE as 45.71 (37.39, 56.75) % and 32.11 (14.73, 75.00) % for PBS and S100β cells respectively. The restoration half-time τ_2 τ_2 was 69.28 (53.08,

92.12) sec for PBS cells and 46.72 (18.68, 113.44) sec for S100β cells. These results confirm that TBOA did not affect the outcome of the DSE (Fig. 9 C and D).

These results demonstrate that the slowdown of glutamate uptake predictably affects PF-EPSC decay time and does not change DSE.

4. Discussion

It was shown previously that activated astrocytes secrete large amounts of S100β [11, 18]. PCs absorb glial S100β in cytoplasmic vacuoles, which leads to changes in their morphology and degeneration [19, 45]. In SCA1 B05 tg mouse model, formation of S100β-containing cytoplasmic vacuoles precedes accumulation of the mutant Ataxin 1 and appearance of the ataxic phenotype [11]. Downregulation of S100β rescues the neurological deficit; therefore, it has been argued that this protein plays the central role in neurodegeneration [46]. We attempted to mimic some of the features of SCA1 by injections of S100β. Mouse S100β was used in order to avoid an immune reaction to a foreign antigen. Using immunohystochemistry we found that the area of S100β deposits spread much further than the actual site of injection and covered 2-3 lobes of the cerebellum. The maximal immunochistochemical signal was seen in lobes 5-7 (Fig. 1C). For imaging we adjusted the brightness of S100β/Alexa 488 fluorescence signal so that to be able to image high concentration S100β without saturation of the system. For this reason, the fluorescence of the endogenous S100β in control mice appears low (Fig. 1A and B). To avoid false positive results when measuring the BG and PC morphology, we used the lobes 6 and 7 which were not directly affected by the injection in all experiments. The excessive amount of S100β altered the morphology of Bergmann glia. Reduction of Bergmann glia cell number (Fig. 2B and F) and processes (Fig. 2D) was accompanied by thickening of the processes (Fig. 2C) and sprouting of new processes in presomatic areas (Fig. 3A and C).

The thickness of molecular layer correlates with the length of PC dendritic tree [47]. To examine the approximate length of PCs dendrites we measured thickness of molecular layer and found that it was significantly reduced by S100β (Fig. 4A and B). Accordingly, soma and dendritic capacitance of PCs also changed significantly (Fig. 4C).

It is interesting that S100β may modulate sodium channels in neurons via Ca^{2+} chelation which affects neuronal sodium channels [48]

which may result in bursting. Kolta with coauthors showed that Ca^{2+} chelation by S100βin intercellular space leads to Na^{+} currents enhancement and make them fire action potentials in bursts, rather than single AP. It is very likely open NMDA receptors and bring more Ca^{2+} inside the neurons [48]. Such a mechanism could lead to Ca^{2+} overload of neurons potentially contributing to neurotoxicity in our model.

Dendritic tree of each PC has thousands of synaptic connections with parallel fibers and 1-2 synapses with climbing fibers [49]. Hence abnormality of dendritic morphology could lead to alteration of basic synaptic transmission, such as EPSC. Our data suggest that S100β did not affect presynaptic glutamate release because it did not significantly change PF EPSC amplitude (Sup. Fig. 2A) and PPF ratio (Fig. 5A). Interestingly, in PCs of 3 week-old SCA1 KI mice, where Ataxin-1 in ubiquitously express, PPF ratio was increased in comparison to its WT littermates (Fig. 6A). We suspect than this phenomenon is due to slowing of glutamate-glutamine cycle machinery because application of FC leads to the same effect in PBS and S100β injected PCs (Fig. 8C). We also show that PF-EPSC rise time is increased in 3 weeks old SCA1 KI mice (Fig. 6B). It is notable that in B05 mice that express mutant Ataxin 1 selectively in PCs rise time was not changed at the same age [50]. Therefore it is likely that changes caused by Ataxin 1 in other cells such as Muller glia are responsible for this effect. Injections of S100β accelerated PF EPSCs decay time (Fig. 5C) which also points to the involvement of the Bergmann glia, which plays the key role in glutamate uptake [51, 52]. Application of TBOA which blocks this uptake, ameliorated the differences between PF-EPSC decay time of PCs in PBS and S100β areas, consistent with this hypothesis (Fig. 9B).

At the same time, astrogliosis and neurodegeneration is characterized by suppression of EAAT1 and EAAT2 function and accumulation of extracellular glutamate leading to excitotoxicity [52]. It highly likely that with age the tendency for prolongation of PF-EPSC decay time SCA1 KI mice (Fig. 6C) will increase and become significant.

Therefore we acknowledge that our S100β injection model may not fully reflect the complex pathological process in Bergmann glia.

Effect of S100β on short-term synaptic plasticity was assessed using DSE. In DSE, strong depolarization leads to Ca^{2+}-dependent release of endocannabinoids from PCs, which retrogradely activate the CB1 receptors on the terminals of PFs [53]. Activation of CB1 inhibits glutamate vesicular release. S100β did not alter PPF ratio which suggests that as such the vesicular release machinery remained intact (Fig. 5A). It is acknowledged that CB may also have direct effects on glia [54] but obviously under our conditions we did not reveal this component.

Our modelling also confirmed that while DSE protocol depolarization was sufficient to decrease PF-EPSC amplitude up to 67-69% of control in S100β and PBS injected mice (Fig. 7A) there was no difference between these two groups. Thus, the induction phase of DSE was not affected by S100 but the recovery after the initial depression was much faster in S100β injected slices. In PBS injected animals amplitude of PF-EPSC was fully restored to the control level at ~100 sec after depolarization, but it only took 60-70 sec in S100β injected group. The predicted speed of restoration by the double waveform fitting (shown by solid and dashed lines on Fig.7A) resulted in the significantly shorter recovery half-time in S100β group compared to the PBS. Endocannabinoids are degraded by fatty acidamide hydrolase and monoacylglycerol lipase. Faster recovery from DSE could be a result of upregulation of these enzymes or simply indicate that S100β affected the process of endocannabinoid release. A possible explanation for the increased PF-EPSC recovery is an increase in extracellular glutamate concentration. In astrocytes CB receptors acts as antagonists of neuronal CB1 receptors and facilitate the neurotransmitter release [54, 41]. Possibly, S100β-activated astrocytes release more glutamate to synaptic cleft after their CB receptors activation by DSE. In such We used FC to evoke metabolic “starvation” of astrocytes. Interestingly DSE (Fig. 8E) in PBS injected areas was sensitive to FC but in S100β it was not. This suggests that the reactive astrocytes after S100β injection

do not respond to endocannabinoids. These data correlates with previous findings [54, 55].

In summary, elevated extracellular S100β leads to reorganization of glia/neuron morphology and disturbs synaptic transmission. Our findings are reminiscent of the early stage of a neurodegenerative process in cerebellar cortex such as seen from 3 old weeks SCA1 mice [25]. The changes in PF-EPSC kinetics reported here were not seen in non-cell selective SCA1 model mice, but take place in PC selective SCA1 model mice, where astrocytes are also affected by the mutant Atxn1 [24 and Fig. 6B]. We hope that our model will assist in better understanding of the role of glia in SCA1 and other diseases which affect cerebellum.

Figure Legends

Figure 1.Localization of S100β in mouse cerebellum.

(A) Confocal image of a cerebellar slice injected with PBS. Left: anti-S100β. Right: anti-GFAP. Scale bar 1mm.

(B) High power confocal image of the zone indicated on (A).Scale bar 100μm.

(C) and (D) – as above but from mice pre-injected with S100β. Scale bar in (C) is 1mm, in (D) 100μm.

Figure 2. S100β alters morphology of Bergmann glia. Microphotographs show anti-S100β and anti-GFAP immunoreactivity in

injected areas from PBS (A) and S100β (B) treated mice. Scale bar 50µm. (C)Summary graph showing the thickness of Bergmann glia processes in µm. *** p< 0.001(D) In a comparison to PBS injected areas, there were significantly fewer Bergmann glia processes per 100µ in S100β injected areas. * p< 0.05 (E) Bergmann glia's processes were more sparse in S100β injected areas. * p< 0.05 (F) Reduction in Bergmann glia cell bodies caused by S100β. ** p< 0.01

Figure 3.Analysis of Bergmann glia process morphology in PBS and S100β-injected mice. *(A) White and black microphotographs of anti-GFAP-labeled areas of cerebellar cortex injected with PBS (left images) and S100β (right images). Light images contain digitally traced Bergmann glia processes generated using ImageJ software. Arrows show the proximal processes which were found more often in S100β injected areas. Scale bar 50µm. (B) Maximum number of processes per Bergmann glia cell did not change between PBS and S100β-injected areas. (C) Sholl analysis for PBS-(open circles) and S100β-injected (closed circles) Bergmann glia cells (number of intersections per 10µm of processes length). ** p< 0.01, ***p<0.001*

Figure 4.S100β alters the PCs morphology of mouse cerebellum.(A) Comparison of immunoreactivity to anti-calbindin (PCs marker), and anti-GFAP in injected areas with PBS (upper panel) and S100β (lower panel). The molecular layer selected by white broken lines. Scale bar 50µm. (B) In comparison to PBS injected areas, the molecular layer was significantly thinner (79,6%) in S100β injected areas. (C) In a comparison to PBS injected areas the capacitance of PCs dendrites and soma measured by voltage-clamp was significantly smaller in S100β injected areas. ** p< 0.01,**<0.01, ***<0.001.

Figure 5.S100β alters PCs electrophysiological properties(A) The summary graph shows the average PFF ratio (2nd amplitude /1st amplitude EPSC) in PCs from PBS and S100β

injected areas, no significant differences found. Below – representative traces of PF-EPSCs (B) The summary graph shows the average rise time of PF-EPSCs in PCs from PBS and S100β injected areas. In comparison to PBS injected areas, rise time was significantly longer in S100β injected areas. Representative traces of PF-EPSCs are shown above. (C) In comparison to PBS injected areas, the decay time was significantly longer in S100β injected areas. The represented traces of PF-EPSCs are above.* $p < 0.05$.

Figure 6. Electrophysiological properties of PCs are altered in 3 weeks old non-cell selective SCA1 model mice. (A) Average PFF ratio (2nd amplitude /1st amplitude EPSC) in PCs from WT and SCA1 KI mice. In comparison to WT PCs, the PPF ratio significantly increased in SCA1 KI mice. Above representative traces of PF-EPSCs. (B) The average rise time of PF-EPSCs in PCs from WT and SCA1 KI mice. In comparison to WT PCs, the rise time was significantly longer in SCA1 KI animals. Representative traces of PF-EPSCs are above. (C) Average decay time of PF-EPSCs in PCs from PBS and S100β injected areas. No significant differences. The represented traces of PF-EPSCs are above. * $p < 0.05$.

Figure 7. S100β disrupts the DSE at PF-PC synapses. (A) Average time course diagram of PF-EPSC amplitudes before and after depolarization. The amplitudes of PF-EPSC were normalized to values before depolarization. The numbers (n) of tested PCs and animals (PCs/animals) are indicated in the graph. Dotted and black lines indicate the double waveform model fit for PBS and S100β injected groups, respectively. Above - representative PF-EPSC traces from PCs from PBS and S100β-injected mice are above the diagram. Time points: before (1) and 50 sec after (2) depolarization. (B) PF-EPSC amplitudes 50 sec after depolarization. In comparison to PBS injected areas, the

PF-EPSC amplitude was significantly bigger in S100β injected areas.* p< 0.05.

Figure 8. Downregulation of astrocytic function by FC affects amplitude and kinetics of PF-EPSC in PF-PC synapses. (A) Time course of PF-EPSC amplitudes before and after 50μM FC application. (B) Averaged PF-EPSC amplitudes 10 min after FC application. Effect of FC was approximately the same in S100β ad PBS injected slices. (C) FC changed significantly PF-EPSC rise time in PBS-injected animals, and slightly increased it after administration of S100β ** p< 0.01, ǂ p<0.05. (D) Representative PF-EPSC traces elicited in PCs from PBS and S100β-injected mice are above the diagram. Time points: before (1) and 50 sec after (2) depolarization. (E) PF-EPSC amplitudes 50 sec after depolarization. In comparison to PBS injected areas, the PF-EPSC amplitude was significantly bigger in S100β injected areas.* p< 0.05 Bergmann glia suppression by FC leads to significant DSE enchantment in PBS injected PCs. ǂ<0.05. (F) Average time course of PF-EPSCs before and after depolarization in slices treated with 50μM FC. Dotted and black lines indicate the double waveform model fit for *PBS* and S100β injected groups, respectively.

Figure 9. Inhibition of glutamate uptake by TBOA alters PF-EPSC kinetic but does not affect endocannabinoid-mediated short term plasticity.

(B) (A) TBOA prolongs EPSP in PBS and S100β-injected tissues (⁎ p<0.05). Note that the baseline tau was reduced after S100β application but the effect of TBOA was comparable to control. TBOA had minimal effect on PPF ratio only in slices from S100β injected animals. * p< 0.05.

(C) Average diagram of PF-EPSC amplitudes before and 30 sec after depolarization. Application of TBOA marked by red line. The amplitudes of PF-EPSC were normalized to values before depolarization.* $p < 0.05$. (D) Representative PF-EPSC traces elicited in PCs from PBS and S100β-injected mice are above the diagram. Time points: before (1) and 30 sec after (2) depolarization. Average time course of PF-EPSCs before and after depolarization in slices treated with TBOA. Dotted and black lines indicate the double waveform model fit for *PBS* and S100β injected groups, respectively.

Supplementary Materials:

Supplementary Figure 1. (A) Confocal image of S100β-injected area (anti-S100β and anti-GFAP staining). The line with two arrowheads illustrates the 100µm length where the parameters of Bergman glia processes were evaluated (B) Confocal image to illustrate staining with anti-Calbindin and anti-GFAP antibodies. The line with two arrowheads illustrates the thickness of cerebellar molecular layer.

Figure S2: Electrophysiological characteristics of PCs (A) The summary graph shows the average PF-EPSC amplitudes in PCs from PBS and S100β injected areas. There was no significant differences between the two groups. The numbers (n) of tested PCs and animals (PCs/animals) are indicated in the graph. (B) Average PF-EPSC amplitudes in PCs from 3 weeks old WT and SAC1 KI mice. Differences between the groups were not significant. The numbers (n) of tested PCs and animals (PCs/animals) are indicated in the graph.

Figure S3: PBS injections do not alter decay time of PF-EPSC.

Acknowledgments: This study was supported by grants «UMNIK» №11950GU/2017 and 5 top 100 Russian Academic Excellence Project at the Immanuel Kant Baltic Federal University.

The authors thank Prof S Kasparov (University of Bristol and BFU) for his critical comments and corrections to the present manuscript.

The authors thank Prof H Hirai for provision of laboratory equipment and SCA1 KI mice.

Conflicts of Interest: The authors declare no conflict of interest.

Author contributions

A.N.S., M.V.S., E.A.P. designed research. A.N.S. performed S100β injections., A.N.S., O.S.B performed the electrophysiological experiments, the morphological analysis. D.A.Y. performed the immunohistochemistry, I.V.P., A.V. performed the Sholl analysis, A.N.S., analysed the data and performed statistics. A.N.S. wrote the paper. All authors approved the final version of the manuscript.

References

1. Sun, Y.-M.; Lu, C.; Wu, Z.-Y. Spinocerebellar ataxia: relationship between phenotype and genotype - a review. Clin. Genet. 2016, 90, 305–314, doi:10.1111/cge.12808.
2. Donato, S. Di; Mariotti, C.; Taroni, F. Spinocerebellar ataxia type 1. Handb. Clin. Neurol. 2012, 103, 399–421, doi:10.1016/B978-0-444-51892-7.00025-5.
3. Tezenas du Montcel, S.; Durr, A.; Rakowicz, M.; Nanetti, L.; Charles, P.; Sulek, A.; Mariotti, C.; Rola, R.; Schols, L.; Bauer, P.; et al. Prediction of the age at onset in spinocerebellar ataxia type 1, 2, 3 and 6. J. Med. Genet. 2014, 51, 479–86. doi:10.1136/jmedgenet-2013-102200.
4. Orr, H.T.; Zoghbi, H.Y. Trinucleotide Repeat Disorders. Annu. Rev. Neurosci. 2007, 30,575–621, doi:10.1146/annurev.neuro.29.051605.113042.

5. Zoghbi, H.Y.; Orr, H.T. Pathogenic Mechanisms of a Polyglutamine-mediated Neurodegenerative Disease, Spinocerebellar Ataxia Type 1. J. Biol. Chem. 2009, 284,7425–7429, doi:10.1074/jbc.R800041200.
6. Bürk, K.; Globas, C.; Bösch, S.; Klockgether, T.; Zühlke, C.; Daum, I.; Dichgans, J. Cognitive deficits in spinocerebellar ataxia type 1, 2, and 3. J. Neurol. 2003, 250, 207–211, doi:10.1007/s00415-003-0976-5.
7. Lim, J.; Crespo-Barreto, J.; Jafar-Nejad, P.; Bowman, A.B.; Richman, R.; Hill, D.E.; Orr, H.T.; Zoghbi, H.Y. Opposing effects of polyglutamine expansion on native protein complexes contribute to SCA1. Nature 2008, 452, 713–8, doi:10.1038/nature06731.
8. Burright, E.N., Clark, H.B., Servadio, A., Matilla, T., Feddersen, R.M., Yunis, W.S., Duvick, L.A.; Zoghbi, H.Y.; Orr, H.T. SCA1 transgenic mice: a model for neurodegeneration caused by an expanded CAG trinucleotide repeat. Cell 1995, 82, 937–48.
9. Duenas, A.M.; Goold, R.; Giunti, P. Molecular pathogenesis of spinocerebellar ataxias. Brain 2006, 129, 1357–1370, doi:10.1093/brain/awl081.
10. Watase, K.; Weeber, E.J.; Xu, B.; Antalffy, B.; Yuva-Paylor, L.; Hashimoto, K.; Kano, M.; Atkinson, R.; Sun, Y.; Armstrong, D.L.; et al. A long CAG repeat in the mouse Sca1 locus replicates SCA1 features and reveals the impact of protein solubility on selective neurodegeneration. Neuron 2002, 34, 905–19.
11. Cvetanovic, M.; Ingram, M.; Orr, H.; Opal, P. Early activation of microglia and astrocytes in mouse models of spinocerebellar ataxia type 1. Neuroscience 2015, 289, 289–299, doi:10.1016/j.neuroscience.2015.01.003.
12. Giovannoni, R.; Maggio, N.; Rosaria Bianco, M.; Cavaliere, C.; Cirillo, G.; Lavitrano, M.; Papa, M. Reactive astrocytosis and glial glutamate transporter clustering are early changes in a spinocerebellar ataxia type 1 transgenic mouse model. Neuron Glia Biol. 2007, 3, doi:10.1017/S1740925X08000185.

13. Cvetanovic, M. Decreased Expression of Glutamate Transporter GLAST in Bergmann Glia Is Associated with the Loss of Purkinje Neurons in the Spinocerebellar Ataxia Type 1. The Cerebellum 2015, 14, 8–11, doi:10.1007/s12311-014-0605-0.
14. Glass, C.K.; Saijo, K.; Winner, B.; Marchetto, M.C.; Gage, F.H. Mechanisms underlying inflammation in neurodegeneration. Cell 2010, 140, 918–34, doi:10.1016/j.cell.2010.02.016.
15. Probert, L.; Akassoglou, K.; Kassiotis, G.; Pasparakis, M.; Alexopoulou, L.; Kollias, G. TNF-alpha transgenic and knockout models of CNS inflammation and degeneration. J. Neuroimmunol. 1997, 72, 137–41.
16. Campbell, I.L.; Abraham, C.R.; Masliah, E.; Kemper, P.; Inglis, J.D.; Oldstone, M.B.; Mucke, L. Neurologic disease induced in transgenic mice by cerebral overexpression of interleukin 6. Proc. Natl. Acad. Sci. U. S. A. 1993, 90, 10061–5.
17. Mosher, K.I.; Wyss-Coray, T. Microglial dysfunction in brain aging and Alzheimer's disease. Biochem. Pharmacol. 2014, 88, 594–604, doi:10.1016/j.bcp.2014.01.008.
18. Rothermundt, M.; Peters, M.; Prehn, J.H.M. S100B in Brain Damage and Neurodegeneration. 2003, 632, 614–632, doi:10.1002/jemt.10303.
19. Vig, P.J.S.; Shao, Q.; Subramony, S.H.; Lopez, M.E.; Safaya, E. Bergmann Glial S100B Activates Myo-inositol Monophosphatase 1 and Co-localizes to Purkinje Cell Vacuoles in SCA1 Transgenic Mice. The Cerebellum 2009, 8, 231–244, doi:10.1007/s12311-009-0125-5.
20. Heizmann, C.W.; Fritz, G.; Schäfer, B.W. S100 proteins: structure, functions and pathology. Front. Biosci. 2002, 7, d1356-68.
21. Marenholz, I.; Heizmann, C.W.; Fritz, G. S100 proteins in mouse and man: from evolution to function and pathology (including an update of the nomenclature). Biochem. Biophys. Res. Commun. 2004, 322, 1111–1122, doi:10.1016/j.bbrc.2004.07.096.

22. Moore, B.W.; McGregor, D. Chromatographic and electrophoretic fractionation of soluble proteins of brain and liver. J. Biol. Chem. 1965, 240, 1647–53.
23. Donato, R. S100: a multigenic family of calcium-modulated proteins of the EF-hand type with intracellular and extracellular functional roles. Int. J. Biochem. Cell Biol. 2001, 33, 637–68.
24. Watase, K.; Weeber, E.J.; Xu, B.; Antalffy, B.; Yuva-Paylor, L.; Hashimoto, K.; Kano, M.; Atkinson, R.; Sun, Y.; Armstrong, D.L.; Sweatt J.D.; Orr, H.T.; Paylor, R.; Zoghbi, H.Y. A long CAG repeat in the mouse Sca1 locus replicates SCA1 features and reveals the impact of protein solubility on selective neurodegeneration. Neuron 2002, 34(6), 905-919.
25. Shuvaev, A.N.; Hosoi, N.; Sato, Y.; Yanagihara, D.; Hirai, H. Progressive impairment of cerebellar mGluR signalling and its therapeutic potential for cerebellar ataxia in spinocerebellar ataxia type 1 model mice. J. Physiol. 2017, 595, 141–164, doi:10.1113/JP272950.
26. Kreitzer, A.C.; Regehr, W.G. Retrograde inhibition of presynaptic calcium influx by endogenous cannabinoids at excitatory synapses onto Purkinje cells. Neuron. 2001, 29(3), 717-27, doi:10.1016/S0896-6273(01)00246-X.
27. Sholl D.A. Dendritic organization in the neurons of the visual and motor cortices of the cat. J Anat. 1953, 87, 387–406.
28. Major, G., Evans, J. D., & Jack, J. J. B. (1993). Solutions for transients in arbitrarily branching cables: II. Voltage clamp theory. Biophysical journal, 65(1), 450-468.
29. Sterratt D.; Graham B.; Gillies A.; Willshaw D. Principles of Computational Modelling in Neuroscience. Cambridge, Cambridge University Press United Kingdom at the University Press, 2011, 404 p.
30. Kaminsky, N.; Bihari, O.; Kanner, S.; Barzilai, A. Connecting Malfunctioning Glial Cells and Brain Degenerative Disorders. Genomics. Proteomics Bioinformatics 2016, 14, 155–165, doi:10.1016/j.gpb.2016.04.003.

31. Liddelow, S.; Barres, B. SnapShot: Astrocytes in Health and Disease. Cell 2015, 162, 1170–1170.e1, doi:10.1016/j.cell.2015.08.029.
32. Khakh, B.S.; Sofroniew, M. V. Diversity of astrocyte functions and phenotypes in neural circuits. Nat. Neurosci. 2015, 18, 942–952, doi:10.1038/nn.4043.
33. Haroon, E.; Miller, A.H.; Sanacora, G. Inflammation, Glutamate, and Glia: A Trio of Trouble in Mood Disorders. Neuropsychopharmacology 2017, 42, 193–215, doi:10.1038/npp.2016.199.
34. Parkin, G.M.; Udawela, M.; Gibbons, A.; Dean, B. Glutamate transporters, EAAT1 and EAAT2, are potentially important in the pathophysiology and treatment of schizophrenia and affective disorders. World J. Psychiatry 2018, 8, 51, doi:10.5498/WJP.V8.I2.51.
35. Machado-Vieira, R.; Manji, H.K.; Zarate, C.A. The Role of the Tripartite Glutamatergic Synapse in the Pathophysiology and Therapeutics of Mood Disorders. Neuroscientist 2010, 15, 525–539, doi:10.1177/1073858409336093.
36. Grewer, C.; Gameiro, A.; Rauen, T. SLC1 Glutamate Transporters The SLC1 transporter family; 2015; Vol. 466; ISBN 0042401313.
37. Swanson, R.A.; Graham, S.H. Fluorocitrate and fluoroacetate effects on astrocyte metabolism in vitro. Brain Res. 1994, 664(1-2), 94-100.
38. Han, J.; Kesner, P.; Metna-Laurent, M.; Duan, T.; Xu, L.; Georges, F.; Koehl, M.; Abrous, D.N.; Mendizabal-Zubiaga, J.; Grandes, P.; Liu, Q.; Bai, G.; Wang, W.; Xiong, L.; Ren, W.; Marsicano, G.; Zhang, X. Acute cannabinoids impair working memory through astroglial CB1 receptor modulation of hippocampal LTD. Cell. 2012, 148, 1039–1050, doi: 10.1016/j.cell.2012.01.037.
39. Navarrete, M.; Araque, A. Endocannabinoids potentiate synaptic transmission through stimulation of astrocytes. Neuron. 2010, 68, 113–126, doi: 10.1016/j.neuron.2010.08.043.

40. Halassa, M.M.; Fellin, T.; Haydon, P.G.; The tripartite synapse: roles for gliotransmission in health and disease. Trends Mol Med. 2007, 13, 54–63, doi: 10.1016/j.molmed.2006.12.005.
41. Rasooli-Nejad, S.; Palygin, O.; Lalo, U.; Pankratov, Y. Cannabinoid receptors contribute to astroglial Ca2+-signalling and control of synaptic plasticity in the neocortex. Philos Trans R SocLond B Biol Sci. 2014, 369(1654), 20140077, doi: 10.1098/rstb.2014.0077.
42. Araque, A.; Li, N.; Doyle, R.T.; Haydon, P.G. SNARE protein-dependent glutamate release from astrocytes.J Neurosci. 2000 Jan 15;20(2):666-73.
43. Beardsley, P.M.; Hauser, K.F. Glial modulators as potential treatments of psychostimulant abuse. Adv. Pharmacol. 2014, 69, 1–69, doi:10.1016/B978-0-12-420118-7.00001-9.
44. Cisneros, I.E.; Ghorpade, A. Methamphetamine and HIV-1-induced neurotoxicity: role of trace amine associated receptor 1 cAMP signaling in astrocytes. Neuropharmacology. 2014, 85, 499–507, doi:10.1016/j.neuropharm.2014.06.011.
45. Vig, P.J.S.; Lopez, M.E.; Wei, J.; D'Souza, D.R.; Subramony, S.H.; Henegar, J.; Fratkin, J.D. Glial S100B Positive Vacuoles In Purkinje Cells: Earliest Morphological Abnormality In SCA1 Transgenic Mice. J NeurolSci Turk 2006, 23(3), 166–174, doi: 10.1901/jaba.2006.23-166.
46. Vig P.J.S.; Hearst S.; Shao Q.; Lopez M.E.; Murphy H.A.; Safaya E. Glial S100B protein modulates mutant ataxin-1 aggregation and toxicity: TRTK12 peptide, a potential candidate for SCA1 therapy. Cerebellum. 2011, 0(2), 254–266, doi: 10.1007/s12311-011-0262-5.
47. Hoxha, E.; Gabriele, R.M.C.; Balbo, I.; Ravera, F.; Masante, L.; Zambelli, V.; Albergo, C.; Mitro, N.; Caruso, D.; Di Gregorio, E.; Brusco, A.; Borroni, B.; Tempia, F. Motor Deficits and Cerebellar Atrophy in Elovl5 Knock Out Mice. Front Cell Neurosci. 2017, 11:343, doi: 10.3389/fncel.2017.00343.

48. Morquette, P.; Verdier, D.; Kadala, A.; Féthière, J.; Philippe, A.G.; Robitaille, R.; Kolta, A. An astrocyte-dependent mechanism for neuronal rhythmogenesis. Nat Neurosci. 2015, 18(6), 844-54, doi: 10.1038/nn.4013.
49. Llinás, R. R.; Sugimori, M. The Electrophysiology of the Cerebellar Purkinje Cell Revisited. The Cerebellum Revisited, 19992, 1, 167-181.
50. Shuvaev, A.N.; Hirai, H. Experience of mGluR1 gene therapy in transgenic models of SCA1 mice. Sovremennyetehnologii v medicine. 2016; 8(4), 141–154, doi: 10.17691/stm2016.8.4.19
51. Mitosek-Szewczyk K.; Sulkowski G.; Stelmasiak Z.; Struzynska L. Expression of glutamate transporters GLT-1 and GLAST in different regions of rat brain during the course of experimental autoimmune encephalomyelitis. Neuroscience 2008, 155(1), 45–52.
52. Lin C.L.G.; Kong Q.; Cuny G.D.; Glicksman M.A. Glutamate transporter EAAT2: a new target for the treatment of neurodegenerative diseases Future Med Chem 2012, 4(13), 1689–1700, doi: 10.4155/fmc.12.122.
53. Kano, M.; Ohno-Shosaku, T.; Hashimotodani, Y.; Uchigashima, M.; Watanabe, M. Endocannabinoid-Mediated Control of Synaptic Transmission. Physiol. Rev. 2009, 89, 309–380, doi:10.1152/physrev.00019.2008.
54. Navarrete M & Araque A (2008). Endocannabinoids mediate neuron-astrocyte communication. Neuron57, 883-893.
55. Gomez-Gonzalo M, Navarrete M, Perea G, Covelo A, Martin-Fernandez M, Shigemoto R, Lujan R, & Araque A (2015). Endocannabinoids Induce Lateral Long-Term Potentiation of Transmitter Release by Stimulation of Gliotransmission. Cereb Cortex25, 3699-3712.