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Effect of propionic acid on the morphology of the amygdala in adolescent male rats and their behavior

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ABSTRACT

Autism spectrum disorder is a group of life-long developmental syndromes, characterized by stereotypic behavior, restricted, communication deficits, cognitive and social impairments. Autism spectrum disorder is heritable state, provided by the mutations of well-conserved genes; however, it has been increasingly accepted, that most of such states are the result of complex interaction between individual's genetic profile and the environment that he/she is exposed to. Gut microbiota plays one of the central roles in the etiology of autism. Propionic acid is one of the most abundant short-chain fatty acids, made by enteric bacteria. Propionic acid has many positive functions and acts as the main mediator between nutrition, gut microbiota and brain physiology. However, increased level of propionic acid is associated with various neurological pathologies, including autism. It is proposed that some types of autism might be partially related with alterations in propionic acid metabolism. The amygdala, the main component of social brain, via its large interconnections with fronto-limbic neural system, plays one of the key roles in social communications, emotional memory and emotional processing. Social behavior is a hot topic in autism research. As to anxiety, it is not the main characteristics of ASD, but represents one of the most common its co morbidities. Several theoretical reasons compatible with amygdala dysfunction have been suggested to account for socio-emotional disturbances in autism.

In the present study, using adolescent male Wistar rats, the effect of acute administration of low dose of propionic acid on social behavior, anxiety-like behavior and the structure/ultrastructure of central nucleus of amygdale was described. In addition to qualitative analysis, on electron microscopic level the quantitative analysis of some parameters of synapses was performed. Behavior was assessed 2, 24 and 48 hours after treatment. The results revealed that even single and relatively low dose of propionic acid is sufficient to produce fast and relatively long lasting (48 h after treatment) decrease of social motivation, whereas asocial motivation and emotional sphere remain unaffected. Morphological analyses of propionic acid-treated brain revealed the reduced neuron number and the increase of the number of glial cells. Electron microscopically, in some neurons the signs of apoptosis and chromatolysis were detected. Glial alterations were more common. Particularly, the activation of astrocytes and microglia were often observed. Pericapillary glia was the most changed. Neuronal, glial and presynaptic mitochondria showed substantial structural diversities, mainly in terms of size and form. Total number of the area of presynaptic profile was significantly decreased. Some axons were moderately demyelinated.

In general, the data indicate that even low dose of propionic acid produces in adolescent rodents immediate changes in social behavior, and structural/ultrastructural alterations in amygdala. Ultrastructural alterations may reflect moderate modifications in functional networks of social brain.

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1. Introduction

Autism spectrum disorder (ASD) is a heterogeneous group of life-long developmental syndromes, characterized by stereotypic behaviors, restricted interests, communication deficits, cognitive and emotional impairments. ASD is a heritable disorder brought about by the mutations of well-conserved genes involved in cell migration, synaptogenesis and network formation. However, it is now believed that ASD is a result of complex gene-environmental interaction (Balaan et al., 2019; Gupta et al., 2014; Kinney et al., 2010; Matsuzaki et al., 2012). ASD's neurobiological basis includes cellular and structural deviations in a variety of brain regions. The diversity of the brain anatomy and the dynamic nature of its development make difficult to clarify the complex mechanisms of ASD. Numerous studies indicate that different brain areas contribute uniquely to autism's impact on cognition and emotions. However, ASD can be best explained as the deficiencies of functional neural networks and not in terms of local dysfunctions (Kana et al., 2017; Lee et al., 2018).

The amygdala, the main component of social brain, has large interconnections with fronto-limbic neural system. Via these connections, amaygdala plays one of the key roles in social communications, gaze, attachment behavior, emotional memory and emotional processing (Dziobek et al., 2010; Eack et al., 2017; Haller, 2018; Paretkar and Dimitrov, 2018). Given that social deficits, abnormal gaze, attachment difficulties and emotional disorders are among the main symptoms of autism, the study of extended amygdala in autistic brain is of special importance (Amaral and Corbett, 2003; Donovan and Basson, 2017; Ecker et al., 2017). Postmortem analysis, functional neuroimaging and experimental studies indicate the abnormal growth patterns, alterations in neuron densities, neuron size, neuronal morphology and other structural changes in autistic amygdala (Avino et al., 2018; Varghese et al., 2017; Velasquez et al., 2017; Weir et al., 2018). In some cases the degree of such alterations positively correlates with the extent of social communication deficits (Avino et al., 2018; Donovan and Basson, 2017; Mitchell et al., 2009). However, due to relatively small number of data and controversial character of some of them, the role of amygdala in socio-emotional impairments in ASD remains inconclusive.

Gut microbiota plays one of the central roles in the etiology of autism. The increased levels of toxin-generating enteric bacteria and short-chain fatty acids, produced by these bacteria are often observed in individuals with ASD (Ding et al., 2017; Vuong and Hsiao, 2017; Wang et al., 2014). Propionic acid (PPA) is one of the most abundant short-chain fatty acids made by enteric bacteria following fermentation of indigestible carbohydrates. PPA has many positive functions. Specifically, PPA acts as the tumor suppressor, regulates enteric neuroendocrine system and several metabolic and anti-inflammatory processes, participates in apoptosis, etc. In addition, PPA represents the main mediator between nutrition, gut microbiota and brain physiology (MacFabe et al., 2007; Xu et al., 2016). However, excessive levels of PPA can produce adverse effects, including developmental delay, mitochondrial dysfunction, oxidative stress, other metabolic and immune reactions (Chapman et al., 2015; MacFabe et al., 2007). Moreover, PPA readily crosses the gut-brain barrier, and affects functional brain networks, provoking the changes in neurotransmitter synthesis, neurotransmission, brain signaling and mitochondrial function (De Almeida et al., 2006; El-Ansary et al., 2018; MacFabe, 2013). Increased level of PPA is associated with various types of neurological disorders, including autism. Recently it was shown that chronic injection of PPA in rodents of different age groups affects social skills, cognitive flexibility and produces some other alterations, compatible to whose, observed in individuals with ASD (El-Ansary et al., 2018; MacFabe et al., 2007; Shultz et al., 2015). Based on these data, it was proposed that some types of autism might be related with the alterations in PPA metabolism (MacFabe, 2013, 2015). In light of this, the use of rodent model of autism, with the purpose to evaluate potential role of microbiota in the pathogenesis of autism is of special importance.

In the present research, we studied the effect of acute administration of low dose of PPA on social behavior, anxiety-like behavior and the structure/ultrastructure of central nucleus of amygdala in adolescent male Wistar rats.

2. Material and methods

2.1. Animals and animal treatment

Male adolescent (P30-35) Wistar rats, weighing 115–125 g, were used. The animals were housed under normal controlled environment (temperature 20–22 °C, humidity 55–60%, light on 07.30–19.30). Standard food pellets and tap water were available. Just before experiments, the rats were randomly divided into experimental and control groups: (i) *Experimental rats* received a single intraperitoneal (i.p.) injection of PPA (Sigma-Aldrich, USA) at a dose 175 mg/kg, pH – 7.4 (*PPA was dissolved in 0.1 M PBS*), (ii) Rats nt the control group were injected with saline. The Committee of Animal Care at I. Beritashvili Center of Experimental Biomedicine and the Committee on Ethics at Ilia State University approved the experimental procedures.

2.2. Animal behavior

2.2.1. Social behavior

Social behavior was assessed 2 h (h) after treatment (15 animals in the group). Conventional three-chamber apparatus, modified to a linear one (45 × 10 × 21 cm), was used (Lee et al., 2016). By this way, the exploration of unnecessary areas was minimized and the number of visits to targets was increased. One-day experiment consisted of two sessions. During first, 5-minute session, the animal, which was placed in the center of empty apparatus, explored the apparatus. During second session, in one corner of the apparatus, translucent acrylic cage (10 × 10 × 10 cm) with stranger rat (social stimulus), and in opposite corner - unfamiliar inanimate object (asocial stimulus) were placed. Experimental rat was allowed to explore the space during 10 min. The rat had free choice to visit social stimulus or non-social stimulus. The behavior was video-recorded. Social propensity was assessed as follows: (i) the number of visits to social stimulus and the number of visits to asocial stimulus and (ii) the time spent in social zone, which is the time, spent in the 4 cm around the cage confining social stimulus, and the time spent in asocial zone – with new object. This measure of behavior has been termed social approach and the preference for unfamiliar object (unsocial stimulus) is thought to reflect social avoidance (Wilson, Koenig, 2014).

2.2.2. Anxiety-related behavior

Emotional sphere, specifically, anxiety-related behavior, was evaluated in elevated plus maze, 2, 24, and 48 h after treatment (Balaan et al., 2019; Sungur et al., 2018; Tanaka et al., 2009). For each case, 15 animals were used. The maze was located in the soundproof room, with constant illumination level. The apparatus consisted of two open and two enclosed arms (45 × 15 × 30 cm each), perpendicularly crossed in the middle, where the central area (14 cm x 14 cm) was located. Each rat was placed in the central area and allowed to move freely for 5 min. Video-tracking system recorded the following parameters: (i) the number and duration of episodes in the central area, (ii) entries in enclosed arms and time spent in enclosed arms, (iii) entries in open arms and time spent in open arms, (iv) the frequency of head movements towards the enclosed arms from open arms, (v) the frequency of head movements towards the open arms from enclosed arms, (vi) grooming activities in open and enclosed arms, (vii) number of vertical standings in enclosed arms, (viii) total number of boluses.

2.2.3. Statistical analysis of behavioral data

Numerical data were calculated by means of statistics package Minitab 17. The two-sample *t*-test was used in order to ascertain the

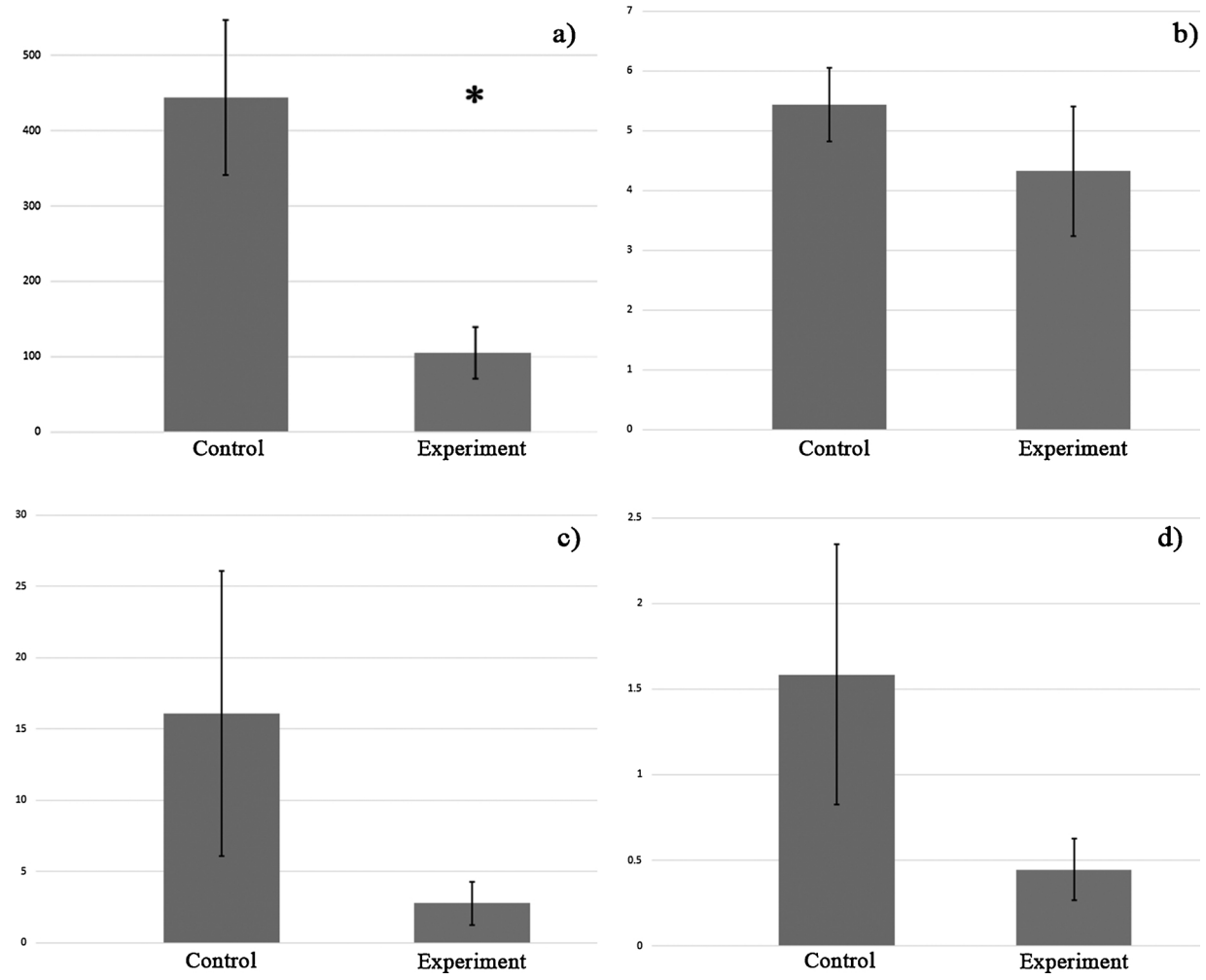

Fig. 1. The assessment of social behavior. The following data are presented: (a) the total time spent in social zone (in close vicinity with unfamiliar rat), (b) the number of visits to social zone (to unfamiliar rat), (c) the total time spent in asocial zone (in close vicinity with unfamiliar object), (d) the number of visits to asocial zone (to unfamiliar object), * $P < 0.05$.

difference between control and experimental groups. The P-value less than 0.05 was considered as statistically significant. The data are presented as a mean ± standard error of the mean (SEM).

2.3. Morphological mygdala

2.3.1. Histological evaluation of neurons and glial cells

Histological analysis of cells was performed 48 h after treatment. Under pentobarbital injection (100 mg/kg), the rats (n = 5 in each group) underwent transcardiac perfusion with heparinized 0.9% NaCl, followed by 500 ml of 4% paraphormaldehyde in 0.1 M phosphate buffer (PB), pH – 7.4 at a perfusion pressure 120 mm Hg. The brains were removed from skull; amygdala was isolated, blocked, frozen, and sectioned in the coronal plane with freezing microtome. 15-micron thick, consecutive coronal sections were collected and placed in 0.1 M PB. Every third section was stained with the Cresyl Violet. Producing the traditional multicolored cells, such staining is suitable for differentiation of various fields, to distinguish principal cells, interneurons and glial cells and to quantify different types of cells. Using anatomical landmarks, totally 10 sections/animal of similar levels of amygdala within and between control and experimental groups were analyzed with optical microscope Leica MM AF. A systemic random sampling was employed and principal and glial cells with distinct nucleus and nucleolus were counted with 2-dimensional counting grid (250 × 250 μm^2).

2.3.1.1. Statistical analysis. To determine whether PPA treatment alters the number of neurons and glial cells, the two-sample *t*-test was used. The level of significance was set as $P < 0.05$. The data are presented as a mean ± standard error of the mean (SEM).

2.3.2. Electron Microscopic Study

Electron microscopic study was performed 48 h after treatment (n = 5, in each group). Following pentobarbital injection (100 mg/kg), the rats to have EM examination of their brains, underwent transcardiac perfusion with 0.9% NaCl followed by 500 ml of 4% parahormaldehyde and 2.5% glutaraldehyde in 0.1 M PB, pH-7.4 at perfusion pressure 120 mm Hg. The brains were sectioned, processed and embedded using conventional procedures, described earlier (Kotaria et al., 2013). 45–50 nm-thick sections were cut with an ultramicrotome Leica EM UC7, picked up on 200-mesh copper grids, double-stained with uranyl-acetate and lead-citrate, and examined with JEM 1400 (JEOL, Japan). From each rat, every fifth section – totally 20 sections per animal were evaluated and the ultrastructure of neurons, glial cells and synapses was described.

2.3.2.1. Quantitative analysis. In addition to qualitative analysis, on each fifth section quantitative processing of presynaptic sectional profile area of axo-dendritic synapses and the total numbers of synaptic vesicles and presynaptic mitochondria in axo-dendritic profiles was perforomed. Finally, a morphometric analysis of the

porosomes, secretory machinery of cells (Cho et al., 2004), was accomplished in presynaptic membranes of saline- and PBS-treated rats, in order to identify any differences in size with regard to diameter of porosome opening and porosome depth.

To measure the surface area of axon terminals and the number of mitochondria within these terminals, on electron micrographs the 180 terminal profiles from PPA-treated rats and 152 profiles for saline-treated rats were outlined. The tracings of axon terminals and the mitochondria they contained were scanned, using the scan plug-in for Adobe Photoshop CS3 and saved as 150 dpi tiff files. The scans were imported into ImageJ software (version 1.44, The National Institute of Mental Health); the areas of profiles were measured and expressed in nanometers. The counting of synaptic vesicles was performed on 75 axon profiles from PPA-treated rats and 75 profiles from saline-treated rats (15 profiles from each animal), using Photoshop. For this purpose, the images of the axon terminals were enlarged onto the computer screen and each vesicle was sequentially marked, using the brush tool. For the analysis of porosome depth and diameter, totally 201 synaptic terminals were observed: n = 100 – in saline-treated rats and n = 101 - in PPA-treated rats. Quantitative data were analyzed using the Image J software. To determine whether PPA affects above mentioned parameters, two-sample *t*-test was used. A P-value less than 0.05 were considered as statistically significant. The results were presented as a mean ± standard error.

3. Results

3.1. Social tendency

Based on data analysis (two-sample *t*-test), 2 h time points, PPA-treated rats spent statistically significant much less time with unfamiliar rats (P = 0.008). No difference was found in the number of visits to unfamiliar rat between PPA and saline-treated animals (P = 0.391). As for the unfamiliar object, no difference between PPA and saline-treated rats was found neither regarding the number of visits to this object (P = 0.172), nor in total time spent near it (P = 0.320) (Fig. 1).

Therefore, experimental rats revealed decreased interest for social stimulus than control rats, and the same interest as control animals to unsocial stimulus. Overall, the data indicate that PPA treatment produces the decrease of social motivation, but asocial motivation remains the same.

3.2. Emotional sphere

In the study, numerous parameters of anxiety-related behavior were evaluated. However, the P-value for all parameters in all terms exceeded 0.05. Such data indicate, that single and relatively low dose of PPA has no effect on anxiety-related behavior of adolescent rats (Table 1).

3.3. Neuronal and glial cell assessment

According two-sample *t*-test (control vs experiment), in the amygdala of PPA-treated rats the number of glial cells was significantly increased (16.2%, P = 0.001) (Fig. 2A). In contrast, slight, but significant decrease of the number of neurons was observed (4.4%, P = 0.03) (Fig. 2B).

3.4. Electron microscopy

Amygdala neurons in PPA-treated brain had mostly normal ultrastructure (Fig. 3A). Only in 9% of observed cells the signs of focal chromatolysis, mild degree of diffuse chromatolysis, swollen cisternae of endoplasmic reticulum, swollen or moderately destructed mitochondria, neurons with osmiophilic cytoplasm or chromatin condensation changes were detected (Fig. 3B,C). Rarely, large dendrites with single dendrotubules and the fragments of dark degenerated, apoptotic cells, partially isolated by astrocyte processes were obsered. (Fig. 3D). The majority of synapses had normal structure; however, in comparing with control, large presynaptic terminals with few synaptic vesicles were often seen. Glial cells were the most changed: swollen and/or proliferating astrocytes and activated microglia (rarely) were detected (Fig. 3D,E,F). Some astrocytes, including pericapillary forms, contained single organelles, and myelin-like, vascuoles or osmiophilic inclusions (Fig. 3E,F). Some axons were moderately demyelinated (Fig. 3B). Absolute majority of mitochondria, including presynaptic forms, had normal ultrastructure, however, substantial structural diversities, particularly in terms of size and form were often observed, rarely, the differences in the energy-transducing inner membrane were detected.

3.4.1. Quantitative analysis of synaptic parameters

In experimental brain, compared to control, the total area of axo-dendritic presynaptic terminals was significantly increased (P < 0.01; by 27%) (Fig. 4). Porosomes were well observed, however statistical difference of their morphological parameters between control and experimental rats was not detected.

4. Discussion

It is generally accepted that autism has many causes and forms, and touches many activities and many regions of the brain. In the present research, we evaluated the consequences of PPA (one of the factors, which provokes autism) on social and anxiety-related behavior and light and electron-microscopic morphology of amygdala in adolescent rats. To the best of our knowledge, the detailed ultrastructural analysis of PPA effect on brain has not been done until now.

Social behavior is a hot topic in autism research. As to anxiety-like behavior, it is not a main characteristic of ASD, but represents one of the most frequent its manifestations. The amygdala is critically involved in both, the regulation of emotions and anxiety-like behavior. Several reasons compatible with amygdala dysfunction have been suggested to account for socio-emotional disturbances in autism (Amaral and Corbett, 2003; Donovan and Basson, 2017; Schoch et al., 2017; Zalla and Sperduti, 2013).

In the normal human colon, the level of PPA is about 20 mM/kg (Al-Lahham et al., 2010). Intraperitneal injection of PPA resulted in a peak in drug level in the brain about 60 min after injection (Brusque et al., 1999). In earlier studies, the scientists described PPA-associated autism-like states in rodents, using chronic injections of substantially larger dose of PPA – 500 mg/kg. Among other changes, the alterations in social and anxiety-like behaviors were detected (Choi et al., 2018; Foley et al., 2014). In the present study, to additionally assess the role of PPA in the development of autism, we described the effects of lower dose of PPA – 175 mg/kg. Our experiments revealed that not only chronic treatment, but also acute i.p. injection of low dose of PPA is sufficient to provide the decrease of social motivation, whereas asocial motivation and anxiety-related behavior remain unaffected. The lower degree of behavioral changes seen in our study, might be due to type of PPA treatment.

Several reasons point to the necessity to evaluate the effects of different doses of PPA on the brain. In particular, recently it was established that the patients who are unable to metabolize PPA are more common than previously thought; many of such patients have cognitive impairments, movement disorders, and seizures (MacFabe, 2013). PPA is usually used in food industry, agriculture and pharmacy; therefore, some diets or pharmaceutical products can easily provoke the increase of PPA level. It is also notable that the role of increased levels of PPA in the pathophysiology of ASD still needs further evaluation. In light of this, the evaluation of neurobiological consequences of various doses of PPA is of special importance. Such approach should improve the

Table 1
The major behavioral activities scored in the elevated plus maze 2, 24 and 48 h after treatment.

2 hours after treatment						
Behavioral Activities		Control rats	PPA treated rats	The Standard Error Control rats	The Standard Error Experimental rats	P Value
Activities in Open arms	*Number of entries in open arms*	0.36	0.78	0.2	0.36	0.340
	Total time spent in open arms	23.73	45.22	23	25	0.532
Activities in Enclosed arms	*Number of entries in enclosed arms*	1.45	1.11	0.25	0.26	0.352
	Total time spent in enclosed arms	267.27	207.22	26	38	0.213
24 hours after treatment						
Behavioral Activities		Control rats	PPA treated rats	The Standard Error Control rats	The Standard Error Experimental rats	P Value
Activities in Open arms	*Number of entries in open arms*	0.36	0.36	0.2	0.2	1.000
	Total time spent in open arms	23.73	31.55	23	21	0.804
Activities in Enclosed arms	*Number of entries in enclosed arms*	1.45	1.09	0.25	0.09	0.193
	Total time spent in enclosed arms	267.27	260.27	26	23	0.840
48 hours after treatment						
Behavioral Activities		Control rats	PPA-treated rats	The Standard Error Control rats	The Standard Error Experimental rats	P Value
Activities in Open arms	*Number of entries in open arms*	0.36	0.91	0.2	0.37	0.214
	Total time spent in open arms	23.73	31.45	23	16	0.784
Activities in Enclosed arms	*Number of entries in enclosed arms*	1.45	1.91	0.25	0.48	0.410
	Total time spent in enclosed arms	267.27	239.72	26	19	0.397

understanding of potential risk factors that lead to the brain abnormalities associated with ASD.

According to the literature, distinct neuronal subpopulations in amygdala differentially participate in social and asocial behaviors (Hong et al., 2014;Rubenstein and Merzenich, 2003). Particularly, GABAergic subpopulation promotes mainly behavior related to aggression, social grooming and mating, whereas glutamatergic subpopulation participates mostly in asocial behaviors, including self-grooming. It is suggested that these two subpopulations act antagonistically, with each inhibiting the behavior regulated by the other (Rubenstein and Merzenich, 2003). The effect of chronic treatment of PPA on both, GABAergic and glutamatergic neuronal subpopulations of amygdala is well known. However, relatively short and low dose of PPA may produce other effects on these cells, causing other behavioral reactions in adolescent rats. Additional biochemical and morphological experiments are needed to define relationship between the structure of amygdala, behavior and changes in neurotransmission.

It is especially notable that in our study, mild neurobehavioral alterations develop in parallel with significant changes in the morphology of amygdala. In particular, light microscopic analyses of PPA-treated brain revealed significant alterations in neuronal and glial populations of amygdala: relatively slight decrease of neurons and the increase of glia. Normally, amygdala has remarkably prolonged development into adulthood. The number of mature neurons in some of the major nuclei of the amygdala increases by 40%, from youth to adulthood (Avino et al., 2018). In adolescent rats, the number of amygdalar neurons also continues to increase (Chareyron et al., 2012). However, in contrast to normal brain, in many regions of autistic brain the amount of different types of neurons is decreased (Avino et al., 2018). Electron microscopy of PPA-treated brain revealed small numbers of residues of dark neurons and neurons with chromatolysis, confirming the loss of amygdala neurons as a result of treatment.

In opposite to neurons, the number of astrocytes in the amygdala of normal brain decreases from 3 weeks to 2 months of age, whereas amygdala volume continues to develop (Chareyron et al., 2012). On the other hand, it is well known that glial cells of social brain are actively involved in ASD. Specifically, close association between ASD and genes related to glial cell activation or genes belonging to immune and

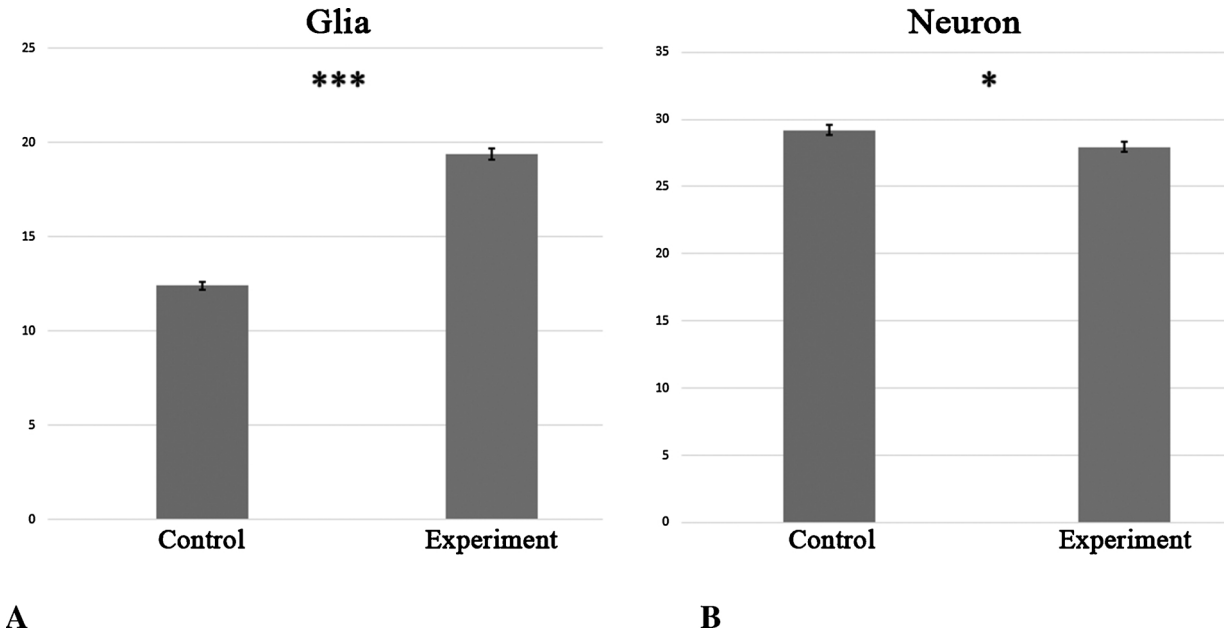

Fig. 2. The analysis of glia (A) and neuron (B) numbers in the central nucleus of amygdala. *P < 0.05, ***P≤0.001.

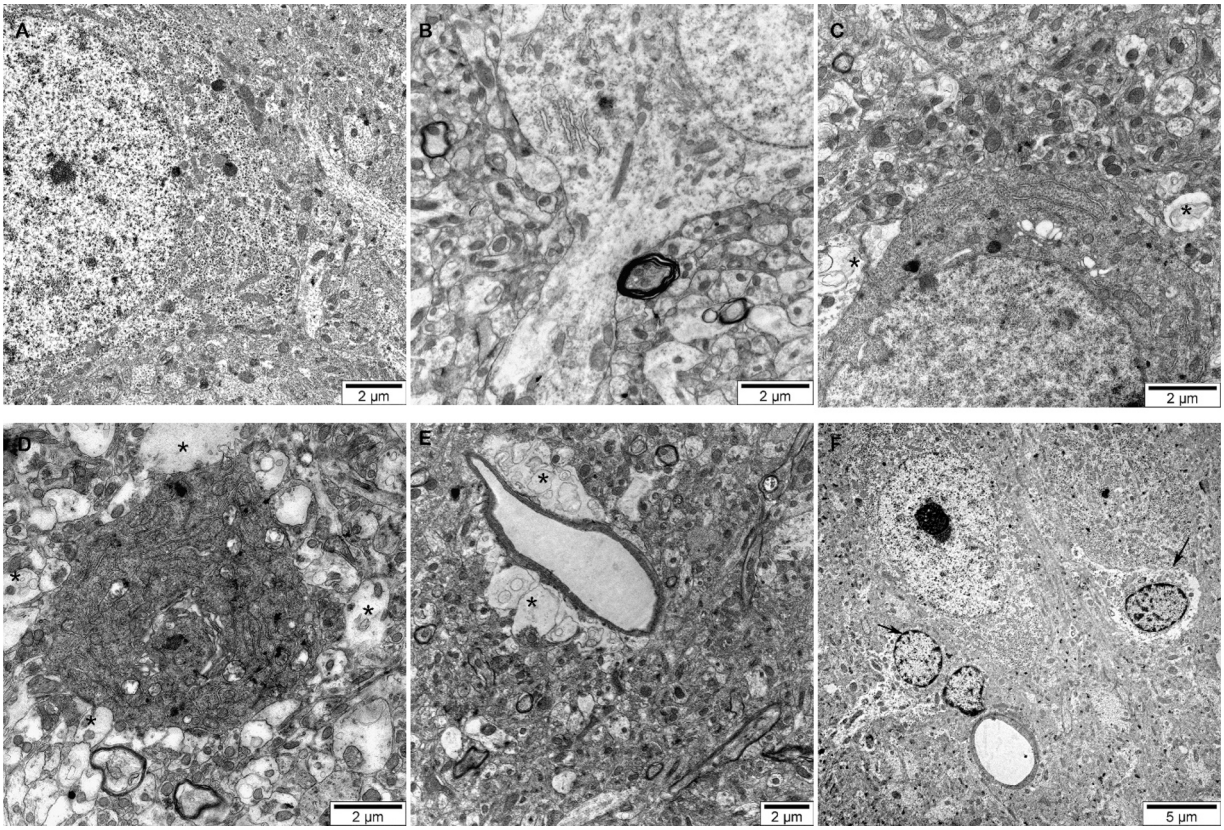

Fig. 3. Representative transmission electron microscopy images of amygdalar neuropil. Control (A) and experimental brain (B,C,D,E,F).
(A) The part of healthy neuron. (B) The part of neuron and axon-hillock with slight chromatolysis. (C) Neuron with highly osmiophilic cytoplasm, lysosomes, and moderately changed organelles, partially surrounded by swollen astrocyte processes. (D) Dark degenerated neuron, fully surrounded by astrocyte processes (indicated with asterisks). (E) Normal micro vessel, fully surrounded by swollen astrocyte processes (indicated by asterisks), forming white perivascular space. (F) Neuropil, with normal micro vessel, neuron with focal chromatolysis of a slight degree, and two activated astrocytes (indicated with arrows). One astrocyte contains few organelles.

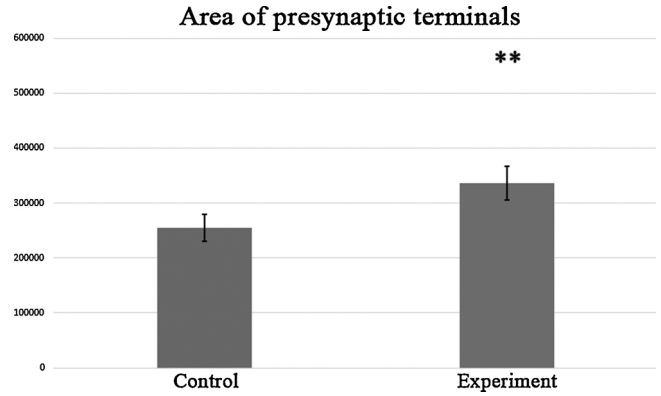

Fig. 4. The effect of PPA treatment on the area of presynaptic terminals in the central nucleus of amygdala of adolescent male rats. **P < 0.01.

inflammatory categories was described (Voineagu, 2012). Clinical, experimental and postmortem analyses indicate to reactive gliosis, glial cell proliferation (Tetreault et al., 2012; Edmonson et al., 2014), microglial activation (Suzuki et al., 2013), or high levels of proinflammatory cytokines in different regions of autistic brain (Li et al., 2009; Wei et al., 2011). Chronic treatment of rodents with PPA is also associated with increased markers for astrocyte and microglia immunoreactivity, demonstrating an innate inflammatory response. Moreover, it was proposed that in the brain PPA is metabolized oxidatively mainly in glia (MacFabe et al., 2007). Special vulnerability of glia to PPA should ultimately lead to structural and functional alterations of these cells, which is demonstrated on electron microscopic level.

It is still unknown whether the inflammation in autism is beneficial or not, or what are the cause/s that provoke activation of glia. According to the most common hypothesis, chronic inflammation plays an important role in the pathogenesis of neurological disorders, related with brain development (Dammann and Leviton, 2004; Gupta et al., 2014; Estes and McAllister, 2015). Various homeostatic mechanisms regulate inflammatory processes, induced by environmental stimuli; astrocytes and microglia play special role in the control of these mechanisms (Barres, 2008; Serhan and Savill, 2005). Under inflammatory conditions, the dysfunction in these mechanisms should provoke higher levels of inflammation, when microglia and astrocytes become reactive. It is also notable that astrocytes and microglia interact with synapse formation, function, plasticity and elimination in developing brain (Chung et al., 2013; Clarke and Barres, 2013; Derecki et al., 2012). Excessive activation of microglia and astrocytes may perturb their capability to modulate the functioning of synapses (Suzuki et al., 2013; Chung et al., 2015).

On electron microscopic level, we revealed substantial structural diversities of mitochondria. Chronic PPA treatment is known to enhance mitochondrial function or, in opposite, to produce a loss of mitochondrial function in a concentration- and time-dependent manner (Frye et al., 2016). Based on our results, we propose, that not only chronic treatment, but low increase of PPA level also, has some effect on neuronal and presynaptic mitochondria. However, to understand the mitochondrial shape changes in response to treatment, high-resolution three-dimensional imaging of mitochondrial structure and the analysis of mitochondrial function is needed (Manella, 2008; Harner et al.,

2016).

Finally, quantitative electron microscopic analysis of PPA-treated brain revealed, that among different synaptic parameters, only the increase in the surface of axo-dendritic synaptic profiles is significantly correlated with the behavioral and ultrastructural impairments displayed by these animals. Such modification should manifest in alteration of synapse function. However, to gain a further understanding of potential changes in the functioning of neural circuits of amygdala, the measurement of additional synaptic parameters is needed (the amount of synaptic vesicles in different vesicle pools, synaptic vesicle number per terminal, length of synaptic junctions, the volume occupied by mitochondria in axon terminals, possible morphological alterations in axo-somatic terminals, etc.).

In general, the data indicate that in rodents even acute administration of low dose of PPA produces significant decrease of social motivation. Behavioral changes are associated with significant modifications in amygdala's structure/ultrastructure. Such alterations should reflect moderate changes in functional networks of amygdala. For further evaluation of the role of propionic acid in the pathogenesis of autism, such data are of special importance.

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Declaration of Competing Interest

None.

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