Original Research Paper

Different Arrangement of Dopamine Receptors/NMDA Receptors Heterocomplexes in the Brain Regions of a Healthy Male, Female and Audiogenic Seizure-Prone Male Rats

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Abstract: Differences in the composition and subcellular localization of heteroreceptors in the brain can trigger central nervous system diseases, including epilepsy and autism. Protein-protein interactions between Dopamine Receptors (DRs) and GLUN2A and GLUN2B subunits are critical mechanisms that regulate dopamine and glutamate coordinated signals. These interactions may be involved in the pathophysiological predisposition of epilepsy. We hypothesized that the specificity of dopaminergic neurotransmission in audiogenic Seizure-Prone (SP) rats could be underlined by the distribution and expression of the N-Methyl-D-Aspartate Glutamate Receptor (NMDAR) and Dopamine Receptor (NMDAR/DR) heterocomplex. We determined the oligomerization of synaptic and extra-synaptic NMDAR subunits with D1 and D2 receptors in the prefrontal cortex and Nucleus Accumbens (NAc) of healthy and epileptic males rats. Considering that the comorbidity of epilepsy and autism is more prevalent in females, we also studied the pattern of interaction. in NMDAR/DR heterocomplex formation in healthy female rats. The association of Dopamine Receptor type 1 (D1R) with the GLUN2A receptor, in the extra-synaptic fraction of epilepsy-prone rats, was lower than that in healthy female and male rats. In contrast, the interaction of D1R with the GLUN2B receptor was higher in the extra-synaptic membranes of epilepsy-prone rats than that in healthy rats. Furthermore, we found that the D2R/GLUN2B ratio was higher in the synaptic NAc fraction of SP and female rats. Therefore, we suggest that the different subunit proportions of the NMDAR/DR heterocomplex in the prefrontal cortex and NAc of male, female, and SP rats can be attributed to their cognitive or emotional flexibility.

Keywords: NMDA Receptor, Dopamine Receptor, Epilepsy, Nucleus Accumbens, Heteroreceptor

Introduction

Recently, new receptor–receptor interactions in heteroreceptor complexes have been described and have been suggested as promising treatment strategies for mental and neurological diseases (Borroto-Escuela *et al.*, 2014). It became clear that the rearrangement of the different receptor homomers into heteroreceptors can lead to the formation of multiprotein complexes with specific adapter proteins to activate a unique downstream regulatory process in the synapse. Such synaptic activity modulations are transformed into specific signaling pathways, ensuring the continuous remodeling of neural circuits that may be involved in drug abuse-induced adaptations as well as in learning and memory processes (Borroto-Escuela and Fuxe, 2019). The interaction of Dopamine Receptors (DRs) with N-Methyl-D-Aspartate Glutamate Receptors (NMDARs) has been studied extensively and their implications in biochemistry, pharmacology, and functioning of the Central Nervous System (CNS) have been described. Several studies have shown that allosteric NMDAR/DR interactions in the

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heterocomplex are essential in cognitive processes and the induction of long-term memory (Floresco, 2015; Wang *et al.*, 2012), which confirms the critical role of these interactions in regulating their functionality (Andrianarivelo *et al.*, 2019; Borroto-Escuela *et al.*, 2018). The recruitment of DRs affects the membrane expression and trafficking of NMDARs, which are important for excitatory neurotransmission and synapse formation. In contrast, the activation of NMDARs modulates the surface expression and signaling of DRs (Navakkode *et al.*, 2007). This reciprocal interaction between Dopamine (DA) and glutamate receptors changes the NMDAR-associated signaling involved in the dopaminergic neuromodulation of cognitive functions (Wise, 2004; Lisman and Grace, 2005). The synaptic/non-synaptic NMDAR/DR heterocomplex location should also be an additional mechanism that regulates DA and glutamate coordinated signals (Cepeda *et al.*, 2009).

It is well-known that DA plays a significant role in the regulation of motor control, learning, reward, and emotions. This monoamine compound, present in various areas of the brain, is altered in many neurological and neuropsychiatric disorders (Wise, 2004; Girault and Greengard, 2004). Among others, the intracellular signaling cascades triggered by limbic DA are involved in long-term epileptogenesis and seizures; its modulating effect depends on the different subtypes of DRs (Beaulieu and Gainetdinov, 2011). Notably, both human and animal studies of limbic epilepsy have shown the opposite effect of signaling mediated by D1-like and D2-like receptors (Bozzi and Borrelli, 2013).

High comorbidity between epilepsy and autism has been suggested by several studies, indicating that similar etiological factors may affect neuropathology (Khetrapal, 2010). Recent investigations have shown that gut microbiome-derived toxins, such as p-cresol, can participate in the pathogenesis of both autism and epilepsy (Tevzadze *et al.*, 2018a, b) and provide expression of differential phenotypes of NMDAR (containing GLUN2B/GLUN2A subunits) in the Hippocampus (Hip) and Nucleus Accumbens (NAc) in healthy and audiogenic Seizure-Prone (SP) rats (Tevzadze *et al.*, 2020). Given that p-cresol affects DA metabolism (Southan *et al.*, 1990) and the activation of DRs triggers a redistribution of GLUN1, GLUN2A, and GLUN2B in synaptic compartments (Dunah *et al.*, 2004), we hypothesized that the specificity of dopaminergic neurotransmission in these brain structures of SP rats, could be highlighted by the distribution and expression of the NMDAR/DR heterocomplex. Therefore, in this study, we determined the oligomerization of synaptic and extra-synaptic NMDAR subunits with D1 and D2 receptors in the prefrontal cortex and NAc of healthy and SP rats. A previous study found changes in the surface expression of NMDAR subunits in the prefrontal cortex and NAc (Tevzadze *et al.*, 2020). Considering that the comorbidity

between epilepsy and autism is higher in females than in males and neurons that control innate social behaviors differ between males and females (Amiet *et al.*, 2008), we also investigated the specificity of NMDAR/DR heterocomplex formation in healthy female rats.

Materials and Methods

Animals

Healthy male and female Wistar rats and male audiogenic SP Krushinski-Molodkina (KM) rats (Poletaeva *et al.*, 2017) (160-180 g weight) were used in the experiments (number of rats in each group = 5). Before euthanization during housing and experiments, the rats were allowed for water and standard laboratory chow, *ad libitum*, and maintained under controlled temperature (21–22°C) and humidity (47±2%), with a 12 h light/dark cycle. The rats were housed in cages (transparent polycarbonate, $595 \times 380 \times 200$ mm), with five animals per cage. The experimental procedures, animal care, and handling were performed in conformity with the European Union (EU) Directive 2010/63/EU for animal experiments. All the experiments were approved by the Institutional Research Projects' Ethics Commission of Ilia State University.

Isolation of Brain Regions and Membrane Fractioning

For the isolation of brain regions, the rats were euthanized and decapitated. After decapitation, the prefrontal cortex and NAc were extracted immediately, then rapidly homogenized in a 5-fold volume of ice-cold buffer (20 mm HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; pH 7.4), 0.02 m sucrose, 5 mm EDTA (ethylene diamine tetraacetic acid) and a cocktail of protease inhibitors (Sigma-Aldrich)) and centrifuged at $1,000 \times g$ for 10 min. To isolate Postsynaptic Density (PSD)-enriched proteins (containing synaptic NMDARs) and non-PSD enriched proteins (containing extra-synaptic NMDARs), we used a previously described method (Martel *et al.*, 2012) with slight modifications according to the following protocol the supernatant, obtained by centrifugation at $1,000 \times g$ was then centrifuged at 4°C at $12,000 \times g$ for 20 min. To extract the extra-synaptic (non-PSD enriched) protein fraction, the pellet was resuspended in a 3-fold volume of solubilization buffer 1(20 mM HEPES, 100 mM NaCl, 1 mm DTT (Dithiothreitol), 1 mm EDTA, 1 mM EGTA (Ethylene Glycol Tetraacetic Acid) and 0.5% Triton X-100; pH 7.2). Solubilization was performed at 4°C for 15 min, followed by centrifugation at $12,000 \times g$ for 30 min. The supernatant was then used in experiments as an "extra-synaptic protein fraction". For solubilization of the synaptic (PSD-enriched) protein fraction, the pellet was resuspended in a 3-fold volume of solubilization buffer 2(20 mm HEPES, 150 mm NaCl, 1 mm

DTT, 10 mm EDTA, 2 mm EGTA, 1% CHAPS (3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate), 0.1% Sodium Dodecyl Sulfate (SDS), 1% Triton X-100, 0.5% IGEPAL® CA-630 and 0.1% BSA (bovine serum albumin; pH 7.4), followed by incubation for 2 h at 4 °C with continuous gentle vortexing. Obtained soluble fractions were centrifuged at $20,000 \times g$ for 30 min and the supernatant was stored and used as a "synaptic protein fraction". All solubilized proteins were stored at -80°C until further experiments.

The total protein amount of both solubilized membrane fractions was determined, using the Micro BCA Protein Assay Kit (cat. no. 23235, Pierce) in quadruplicate according to manufacturer guidelines. All the samples were stored at -80°C until analysis.

Western Blotting

Aliquots of the solubilized membrane fractions (containing ≈30 μg of total protein) were dissolved in equal volumes and loaded on SDS gels (4-12%) and electrophoresed. The separated proteins were then transferred onto 0.45 μm nitrocellulose membranes using electroblotting. Membranes were stained with Ponceau S solution to confirm correct sample loading and efficient protein transfer. Subsequently, the membranes were blocked with 5% BSA in Tris-buffered saline with 0.1% Tween-20 (TBST) and incubated for 1 h with antibodies against GLUN2B (sc-390094), GLUN2A (sc-3655R97), D1DR (sc-33660) and D2DR (sc-5303) at a dilution of 1:1000 (Santa Cruz, USA). After incubation, the membranes were washed in TBST and probed with species-appropriate peroxidase-conjugated secondary antibodies at 20°C for 1 h. After further washing in TBST, a chemiluminescent substrate (Santa Cruz, USA) was used for standard immunochemical procedures. The blots were exposed to audio radiograph films (Amersham), Obtained films were then digitalized by photo equipment. and their intensities quantified using Image Lite Studio software version 5.2.5 (Li-Cor).

Immunoprecipitation

Aliquots of protein fractions with similar protein amounts were incubated overnight at 4°C with GLUN2 antibody-bound protein A/G-Agarose beads. After washing, the protein A/G-Agarose pellets were resuspended in 100 mm glycine at a pH of 3.0 for 10 min, and then a pretitrated volume of 1.0 M Tris (pH 9.5) was added to adjust the pH to 7.4. Protein complexes in the supernatants ($2,500 \times g$, 10 min) were analyzed by western blotting.

Statistical Analyses

Optical density values for GLUN2B, GLUN2A, D1DR, and D2DR were analyzed with a one-way analysis of variance. Groups were compared by planned comparisons, using two-tailed t-tests. Statistics 9 (Analytical Software, Tallahassee, USA) was used for all statistical analyses.

Results

First, the expression of GLUN2B and GLUN2A subunits in the synaptic and extra-synaptic fractions of the prefrontal cortex was determined in SP, healthy male and female rats. Our results showed that the levels of protein surface expression in extra-synaptic as well as in synaptic GLUN2A and GLUN2B subunits in the prefrontal cortex of SP rats were the same in all three animal groups and did not differ significantly (Fig. 1A).

Next, the contents of D1/GLUN2B and D1/GLUN2A heterocomplexes were determined in the synaptic and extra-synaptic fractions of the prefrontal cortex, in all three animal groups by immunoprecipitation. We found that the extra-synaptic fraction of the prefrontal cortex of KM rats contained more D1R associated with the GLUN2B subunit (Fig. 2A), than in healthy male and female rats. Interestingly, the association of D1R with GLUN2A was lower in the cortex extra-synaptic membranes of KM and female rats, than in healthy male rats (Fig. 2B). No significant changes were observed in the D1R/GLUN2A and D1R/GLUN2B association in both synaptic fractions of cortex membranes (Fig. 2). Furthermore, the protein-protein association of D2R with the NMDAR subunits (GLUN2A and GLUN2B) was determined (Fig. 3). A significantly higher degree of association between D2R and GLUN2B was only found in the synaptic fraction of female rats. In contrast, the interaction of these receptors in the extra-synaptic heterocomplex was not changed in either of the male cortexes, but it was slightly decreased in female rats (Fig. 3A). The association of D2R with GLUN2A did not differ significantly in synaptic or extra-synaptic fractions in all animal groups (Fig. 3B). We performed a quantitative analysis of both types of DRs in synaptic and extra-synaptic neurons to exclude the effect of DR overexpression on the degree of binding to the NMDA glutamate receptor fractions. Experimental results showed no statistically significant differences between the three groups for the DR membrane expression subtypes in the synaptic fraction (Fig. 4A). Elevated D1R expression may result from increased levels of the total extra-synaptic D1 receptor. Interestingly, increased surface expression of synaptic D2 receptors was also observed in female rat brains (Fig. 4B).

An imbalance in the ratio between excitation and inhibition is one of the most important parameters of epileptic brains (Engel *et al*., 2008). Recent evidence shows that neuromodulators, such as DA, can affect the excitation/inhibition ratio by inducing some glutamatergic neuron activity changes. This interaction plays a critical role in controlling neuronal activity during seizures (Gonzalez-Islas and Hablitz, 2003; Slaght *et al*., 2002). Significant changes occurred in different aspects of dopaminergic transmission in humans and laboratory animals as shown in previous studies (Bozzi and Borrelli, 2013; Starr, 1996). In

addition to significant dopaminergic system changes (in release, metabolism, and/or receptor binding of DA) following epileptic seizures, dopaminergic neurons also modulate synaptic plasticity (Hansen and Manahan-Vaughan, 2014). It was shown that the modulation of DA signaling most critically affects episodes involving the limbic system. Brain regions receiving afferents from the mesolimbic dopaminergic pathway express different types of DA receptors (Bozzi and Borrelli, 2013; 2006; Bozzi *et al.*, 2011). Both the hippocampus and Prefrontal Cortex (PFC) receive DA innervation from the Ventral Tegmental Area (VTA) and the NAc through multiple receptors, among which D1R and D2R are the most abundant. Thus, the protein-protein association between D1/D2R and GLUN2A/GLUN2B was determined in the next series of experiments. However, the expression of NMDAR subunits in the NAc of the three experimental animal groups was assayed.

It was found that the membrane expression of the extra-synaptic GLUN2A subunit in the NAc of KM and healthy female rats was lower than that in healthy male rats (Fig. 5B). In contrast, extra-synaptic and synaptic GLUN2B expression did not differ significantly between groups (Fig. 5A).

The degree of protein-protein association between D1R and NR subunits differed significantly in a synaptic and extra-synaptic fraction of NAc. The level of D1R in the heterocomplex D1/GLUN2A in KM synaptic and extra-synaptic fractions was lower than in healthy female and male rats (Fig. 6B), whereas the levels of D1R in the heterocomplex D1/GLUN2B was higher in the KM rats (Fig. 6A). Interestingly, the D1R/GLUN2A ratio was higher in female rats than in

male and KM rats, while the binding of D1R to the GLUN2B subunit was much lower in female rats. It should be noted, that the synaptic D1R/GLUN2B heterocomplex in male NAc fractions contains a minimal amount of D1, compared to the KM and female rats.

The level of D2R, either in the extra-synaptic heterocomplex D2R/GLUN2A or in the extra-synaptic heterocomplex D2R/GLUN2B, in the NAc of experimental animals, did not differ significantly between groups (Fig. 7). In contrast, a high D2R content was found in the synaptic heterocomplex D2R/GLUN2B, in the NAc of KM and female rats (Fig. 7A). Moreover, a more significant D2R was observed in the synaptic heterocomplex D2/GLUN2A in female rats, than in healthy male and KM rats.

Therefore, experiments revealed that in the cortex of KM rats, the association of D1R with the GLUN2A receptor in the extra-synaptic fraction was lower, but the interaction of the same D1 receptor with GLUN2B was higher compared to respective fractions of healthy female and male rats. The content of D1R in the heterocomplex D1/GLUN2A in the NAc of KM rats was lower than in the healthy female and male rats, whereas the levels of D1R in the heterocomplex D1/GLUN2B were higher in the KM rats in both synaptic and extra-synaptic membranes. The D1R/GLUN2A ratio was higher in female rats than in male and KM rats, while the interaction of D1R to the GLUN2B subunit was much lower in the NAc of female rats. The synaptic D1R/GLUN2B heterocomplex of healthy male NAc contains a minimal amount of D1, compared to the corresponding fraction OFE KM and healthy female rats.
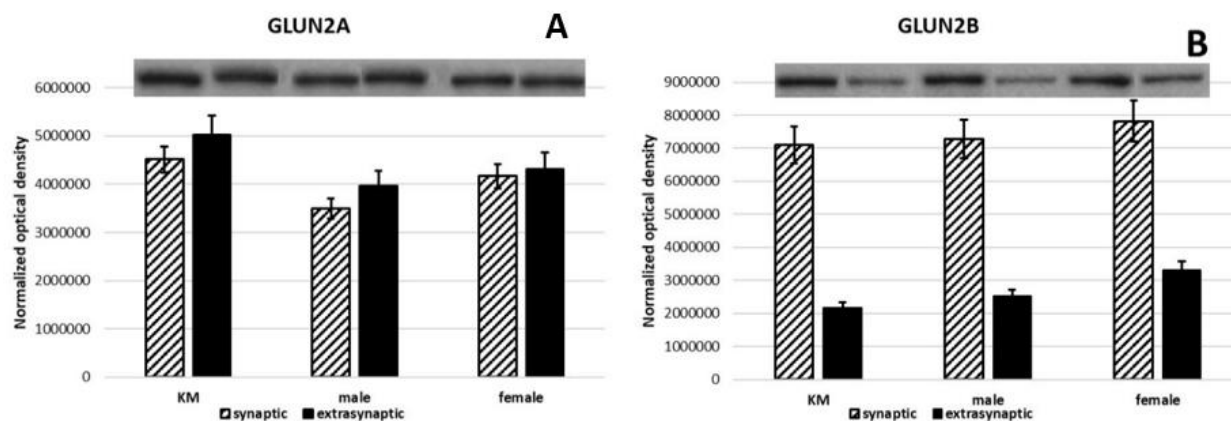

Fig. 1: Protein expression of GLUN2A (A) and GLUN2B (B) in the synaptic and extra-synaptic membrane fractions in the prefrontal cortex of healthy male, female, and KM rats, as measured by western blotting. Samples of solubilized membrane fractions with equal amounts of total protein were separated on 4-12% gradient gels. After transferring to 0.45 μm nitrocellulose, the blotted bands were immunodetected with specific primary antibodies and then visualized with peroxidase-labeled secondary IgG antibodies. The bands (one is displayed over the respective chart) were acquired and their intensities were quantified using Image Lite Studio software. Data are normalized and expressed as mean optical density ± Standard Error of the Mean (SEM) ($p<0.05$)
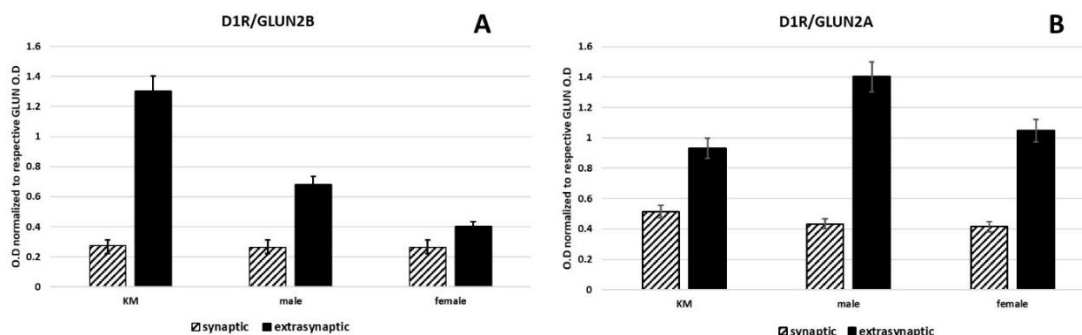
Fig. 2: Amount of DR1 protein associated with GLUN2B (A) and GLUN2A (B) in the synaptic and extra-synaptic membrane fractions of the prefrontal cortex of healthy male, female, and KM rats, as measured by western blotting. Samples of solubilized membrane fractions with equal amounts of total protein were immunoprecipitated by the antibody of the NMDAR subunit and separated on 4-12% gradient gels. After transferring to 0.45 μm nitrocellulose, the blotted bands were immunodetected with specific primary antibodies and then visualized with peroxidase-labeled secondary IgG antibodies. The bands were acquired and their intensities were quantified using Image Lite Studio software. Data are expressed as mean ± SEM and calculated relative to the optical density of GLUN2 in the respective fraction ($p < 0.05$)
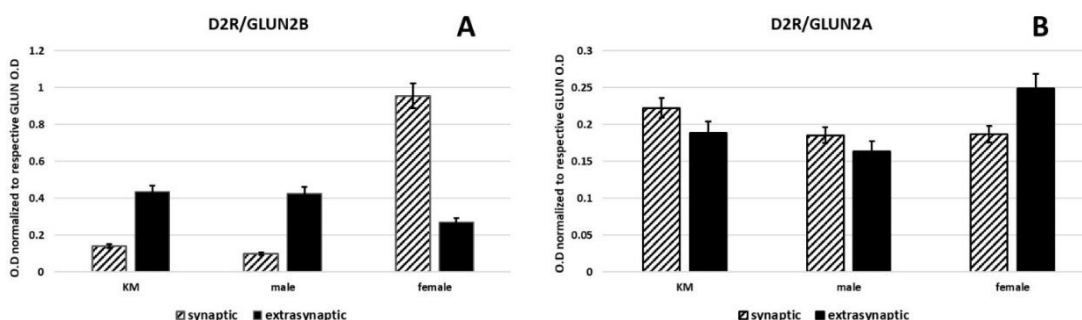

Fig. 3: Amount of DR2 protein associated with GLUN2B (A) and GLUN2A (B) in the synaptic and extra-synaptic membrane fractions of the prefrontal cortex of healthy male, female, and KM rats, as measured by western blotting. Samples of solubilized membrane fractions, with equal amounts of total protein, were immunoprecipitated by the antibody of the NMDAR subunit and separated on 4-12% gradient gels. After transferring to 0.45 μm nitrocellulose, the blotted bands were immunodetected with specific primary antibodies and then visualized with peroxidase-labeled secondary IgG antibodies. The bands were acquired and their intensities were quantified using Image Lite Studio software. Data are expressed as mean ± SEM and calculated relative to the optical density of GLUN2 in the respective fraction ($p < 0.05$)
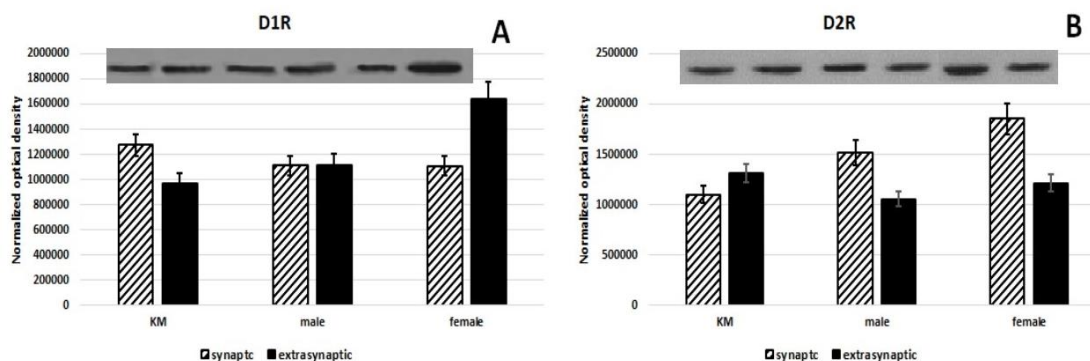

Fig. 4: Protein expression of DR1 (A) and DR2 (B) in the synaptic and extra-synaptic membrane fractions of the prefrontal cortex of healthy male, female, and KM rats, as measured by western blotting. Samples of solubilized membrane fractions, with equal amounts of total protein, were separated on 4-12% gradient gels. After transferring to 0.45 μm nitrocellulose, the blotted bands were immunodetected with specific primary antibodies and then visualized with peroxidase-labeled secondary IgG antibodies. The bands (one is displayed over the respective chart) were acquired and their intensities were quantified using Image Lite Studio software. Data are normalized and expressed as mean optical density ± SEM ($p < 0.05$)
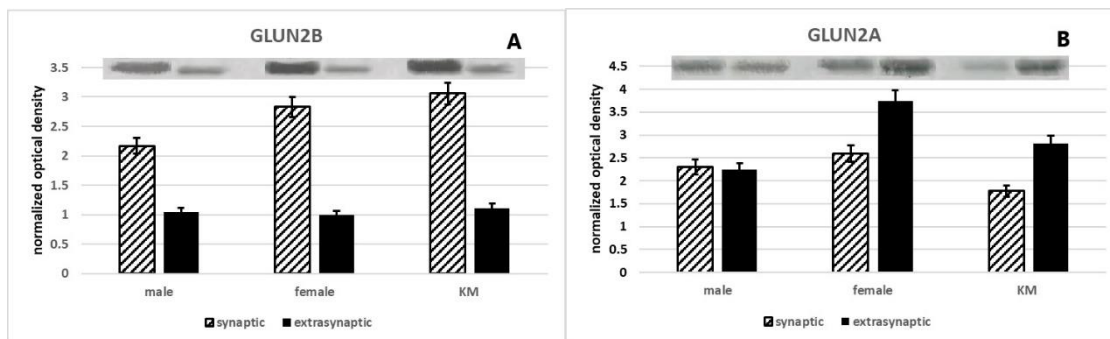
Fig. 5: Protein expression of GLUN2B (A) and GLUN2A (B) in the synaptic and extra-synaptic membrane fractions of nucleus accumbens of healthy male, female, and KM rats, as measured by western blotting. Samples of solubilized membrane fractions, with equal amounts of total protein, were separated on 4-12% gradient gels. After transferring to 0.45 μm nitrocellulose, the blotted bands were immunodetected with specific primary antibodies and then visualized with peroxidase-labeled secondary IgG antibodies. The bands (one is displayed over the respective chart) were acquired and their intensities were quantified using Image Lite Studio software. Data are normalized and expressed as mean optical density ± SEM ($p<0.05$)
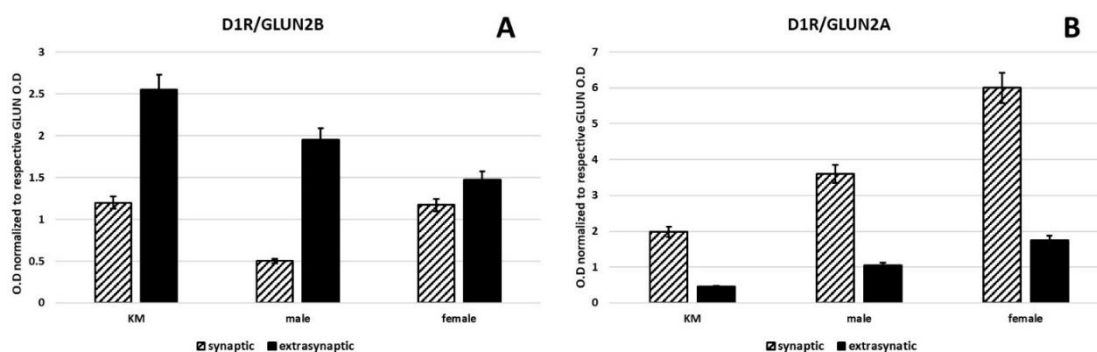

Fig. 6: Amount of DR1 protein associated with GLUN2B (A) and GLUN2A (B) in the synaptic and extra-synaptic membrane fractions of nucleus accumbens of a healthy male, female, and KM rats, as measured by western blotting. Samples of solubilized membrane fractions with equal amounts of total protein were immunoprecipitated by the antibody of the NMDAR subunit and separated on 4-12% gradient gels. After transferring to 0.45 μm nitrocellulose, the blotted bands were immunodetected with specific primary antibodies and then visualized with peroxidase-labeled secondary IgG antibodies. The bands were acquired and their intensities were quantified using Image Lite Studio software. Data are expressed as mean ± SEM and calculated relative to the optical density of GLUN2 in the respective fraction ($p<0.05$)
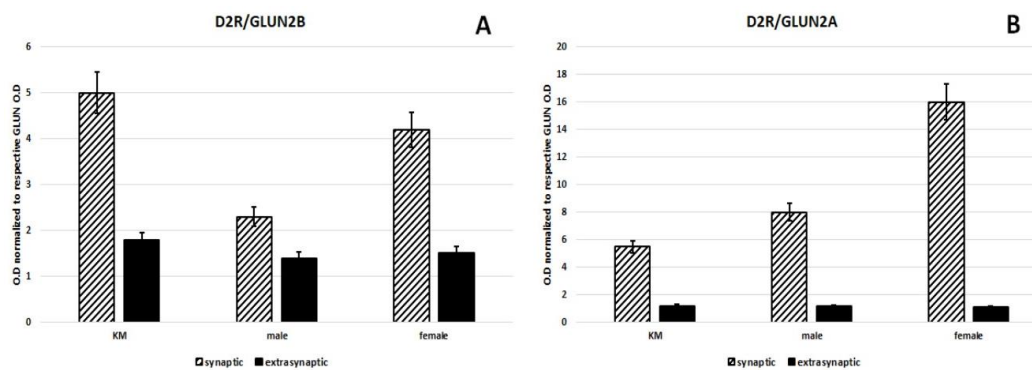

Fig. 7: Amount of DR2 protein associated with GLUN2B (A) and GLUN2A (B) in the synaptic and extra-synaptic membrane fractions of nucleus accumbens of a healthy male, female, and KM rats, as measured by western blotting. Samples of solubilized membrane fractions, with equal amounts of total protein, were immunoprecipitated by the antibody of the NMDAR subunit and separated on 4-12% gradient gels. After transferring to 0.45 μm nitrocellulose, the blotted bands were immunodetected with specific primary antibodies and then visualized with peroxidase-labeled secondary IgG antibodies. The bands were acquired and their intensities were quantified using Image Lite Studio software. Data are expressed as mean ± SEM and calculated relative to the optical density of GLUN2 in the respective fraction ($p<0.05$)

Discussion

Synaptic plasticity is an important pathophysiological factor in epilepsy (Bozzi *et al.*, 2011). Dopaminergic neurons, arising in the limbic system, play a significant modulatory role in controlling seizures and may be involved in long-term epileptogenesis (Bozzi and Borrelli, 2013). The brain areas receiving afferents from the mesolimbic dopaminergic pathway express different DA receptors, which may have an opposite role in limbic epilepsy. The NAc, hippocampus, and PFC receive DA innervation from the VTA through various DRs (Floresco, 2015; Lisman and Grace, 2005; Pirot *et al.*, 1992). Stimulation of these receptors regarding glutamatergic activity has the opposite effects: D2R activation inhibits NMDAR currents in medium efferent neurons (Wang *et al.*, 2012), whereas activating D1R facilitates the surface expression and function of NMDAR and enhances long-term potentiation (Li *et al.*, 2010). Reciprocally, the activation of NMDARs modulates D1R surface expression and signaling (Navakkode *et al.*, 2007). This bidirectional interaction between DRs and NMDAR-associated current results in changes in receptor content and trafficking in a membrane (Lee *et al.*, 2002; Fiorentini *et al.*, 2003). Notably, the activation of D1R, but not D2R, is essential for NMDAR-dependent long-term potentiation in hip–PFC connections, and this mechanism possibly underlies the transfer and storage of contextual information (Gurden *et al.*, 2000). Considering these observations, we hypothesized that the formation and reconstruction of the DR/NMDAR heterocomplex may modulate the sensitivity to limbic epilepsy.

Based on this supposition, the content and ratio of D1 and D2 dopamine receptors and GLUN2 subunits of NMDAR in D1R/GLUN2A, D1R/GLUN2B, D2R/GLUN2A, and D2R/GLUN2B heterocomplexes were determined in the synaptic and extra-synaptic fractions of the PFC and NAc of healthy male and female rats, as well as in the audiogenic SP rats (KM rats). We found that in the cortex of KM rats, the association of D1R with the GLUN2A receptor was lower than that in the extra-synaptic fraction of healthy female and male rats. In contrast, the interaction of the same D1 receptor with GLUN2B was higher in the extra-synaptic membranes of KM rats. Besides, we found that in the NAc of KM rats, the content of D1R in the heterocomplex D1/GLUN2A was lower than in the healthy female and male rats, whereas the levels of D1R in the heterocomplex D1/GLUN2B were higher in the KM rats. A similar distribution of receptors in the heterocomplex was observed in both synaptic and extra-synaptic membranes. Interestingly, the D1R/GLUN2A ratio was higher in female rats than in male and KM rats, while the binding of D1R to the GLUN2B subunit was much lower in the NAc of female rats. It is essential to mention that the synaptic D1R/GLUN2B heterocomplex of healthy male

NAc contains a minimal amount of D1, compared to the KM and healthy female rats.

Considering that the comorbidity of epilepsy and autism is more prevalent in females (Amiet *et al.*, 2008; Fombonne, 2005), we analyzed the levels of associated DR/NMDAR subunits in the heterocomplex, in both healthy male and female rats. We found that in the cortex, the ratio of D1R/GLUN2A in the heterocomplex does not differ significantly between healthy male and female animals at both synaptic and extra-synaptic membranes. However, in the NAc, the association of D1 and GLUN2A subunits was higher in female rats than in male rats, especially in the synaptic membranes. In the synaptic membranes of the NAc of healthy female rats, the content of D2R in the heterocomplex D2R/GLUN2B is also increased, compared to that in males.

Furthermore, we found that the D2R/GLUN2B ratio was higher in the synaptic NAc fraction of KM and female rats. Taking into account that the association of GLUN2B with D2R in the heterocomplex decreases NMDAR-dependent activity and considering that the synaptic activation of GLUN2B is involved in the long-term drug-associated memory effects (Xu *et al.*, 2012), we could speculate that contextual interaction between novelty and reward processing within the mesolimbic system was lower in female and KM rats than in healthy male rats. The enhanced physical interaction of D2R with GLUN2B can disrupt Ca^{2+} Calmodulin-dependent Protein Kinase II (CaMKII) association with GLUN2B and inhibits NMDAR-mediated currents in the NAc (Liu *et al.*, 2006). Interestingly, the same high synaptic D2R/GLUN2B ratio was only observed in the cortex of healthy female rats, whereas this heterocomplex was not changed in healthy male and KM rats. However, it can be proposed that an increased density of synaptic D2R in the NAc of female and KM rats can increase motivated behavior by disinhibiting the ventral pallidum (Gallo *et al.*, 2018). Overall, the data suggest that motivational, memory and cognitive dissimilarities may be prevalent between these animal groups, based on the generalization that enhanced content of D2Rs in the PFC and NAc are thought to play important roles in behavior, working memory, and cognitive flexibility (Zhang *et al.*, 2007).

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Author's Contributions

Gigi Tevzadze: Proposed main hypothesis, designed main tasks, and participated in paper writing.

Elene Zhuravliova: Designed the main stages of the research plan and organized the study, participated in data analysis.

Natia Okriashvili: Carried our main experiments with brain structure extraction and subcellular fractioning, and participated in western blotting experiments.

Nana Narmania: Carried our immunoprecipitation and western blotting, and made densitometry evaluation and data analysis.

Tamar Barbakadze: Made statistical analysis of obtained data, and prepared visual part of the paper.

David Mikeladze: Made interpretation of obtained data, prepared introduction of obtained data, prepared introduction and discussion part of the paper.

Ethics

The authors declare their responsibility for any ethical issues that may arise after the publication of this manuscript.

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