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Posted Date: 20 September 2023

doi: 10.20944/preprints202309.1353.v1

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D1-like and D2-like Dopamine Receptor of Rat Prefrontal Cortex: Impacts of Genetic Generalized Epilepsies and Social Behavioral Deficits

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Abstract: The involvement of the prefrontal cortical dopaminergic system in psychopathology of epilepsies and comorbid conditions such as autism spectrum disorder (ASD) still needs to be explored. We used autoradiography to study the D1-like (D1DR) and D2-like (D2DR) receptor binding density in the prefrontal cortex of normal Wistar rats and Wistar-derived strains with generalized convulsive and/or nonconvulsive epilepsies. WAG/Rij rats served as a model for non-convulsive absence epilepsy, WAG/Rij-AGS as a model of mixed convulsive/non-convulsive forms, and KM strain was a model for convulsive epilepsy comorbiding with ASD-like behavioral phenotype. Prefrontal cortex of rats with any of epileptic pathology studied, demonstrated profound decreases in binding densities to both D1DR, D2DR; the effects were localized in the primary and secondary anterior cingulate cortexi, and adjacent regions. The local decreased D1DR and D2DR binding densities were independent (not correlated) from each other. The particular group of epileptic rats with ASD-like phenotype (KM strain), displayed changes in the lateral prefrontal cortex and adjacent regions: D1DR were lowered, but those to D2DR elevated, in anterior dysgranular insular cortex and adjacent regions. Thus, epilepsy-related changes in the dopaminergic system of rat archeocortex were localized in the medial regions, whereas ASD-related candidates were seen in the lateral aspects. The findings point to putative local dopaminergic dysfunctions, associated with generalized epilepsies and/or ASD.

Keywords: dopamine; D1-like dopamine receptors; D2-like dopamine receptors; prefrontal cortex; generalized epilepsy; absence epilepsy; audiogenic epilepsy; animal model; autism spectrum disorder; phenotype

1. Introduction

The existence and normal functioning of the brain make us not only living beings but also conscious, thinking and creative creatures, make us who we are, how we live, feel, perceive and analyze information, draw conclusions and plan, respond, behave and communicate, ill and die. One of the features that ensures the brain functioning is mediatory systems, the system of chemical messengers and their specific receptors. Each of neurotransmitters plays unique complex role in the brain orchestra. Leaders of that orchestra are glutamate, the predominant excitatory neurotransmitter, and GABA, the inhibitory one [1]. But, as in real orchestra, other "players" are also important. A particular modulator, dopamine, is now one of the most mentioned in literature as a substance of pleasure and reward.

Dopamine participates in a wide spectrum of brain processes such as alertness, attention, cognition, and mood modulation. Studies of brain dopaminergic system are possible by a variety of different electrophysiological, neurochemical, genetic, neuropharmacological and molecular imaging

 doi:10.20944/preprints202309.1353.v1

techniques. One of the possible ways to explore the state of the dopaminergic system is to analyze the binding density to dopamine receptors.

It is traditionally accepted that dopamine neurotransmission occurs through the six types of dopamine receptors, sub-classified as the D1-like (D1 and D5) (D1DR) and the D2-like (D2short/long, D3, and D4) (D2DR) receptors [2,3]. For quite some time the ability of dopamine receptors to form complexes with dopamine receptors of other types, and receptors of different signaling ligands, was revealed [4]. It leads to a need for simultaneous study of at least more than one type of dopamine receptor at the same time. The impaired activity of dopamine and its receptors is a pathological factor for many human brain illnesses, the most frequently mentioned of which are Parkinson's disease and schizophrenia. But there are growing evidences that, even though main neurotransmitters involved in epileptogenesis are glutamate and gamma-aminobutyric acid (GABA), the imbalance of neuromodulators, such as dopamine, can play an important role in the development both epileptic seizures and comorbid conditions [5]. For experimental approaches to study epilepsy and its comorbidities, one needs animal models for this human neuropathology. Laboratory rodents are widely used to study neurophysiology and neurochemistry of human neurological diseases.

We have a prolific opportunity to study several related rat strains with different forms of genetic generalized epilepsies. All of these strains were bred from normal rats of Wistar strain. First, WAG/Rij strain, is recognized as one of the best models of absence epilepsy. Absence seizures (AbS) manifest as attacks with generalized spike-wave EEG discharges and behavioral arrests [6–8]. Second group is, in fact, a sub-strain of WAG/Rij, WAG/Rij-AGS, whose rats demonstrate both absence non-convulsive seizures and convulsive audiogenic seizures(AGS) in respond to a sound provocation (audiogenic epilepsy) [9,10]. This sub-strain can be used as a model of the mixed form of epilepsy. Last but not least, a strain to compare is KM (Krushinsky-Molodkina) strain, whose rats are prone to severe audiogenic convulsions and serve as a model for human temporal lobe epilepsy [11–13]. That it, the group was characterized with a pure convulsive form of epilepsy. Thus we are able to compare three different models of genetic generalized epilepsies: convulsive, non-convulsive and mixed and refer to Wistar strain as controls. Since rats of all these epileptic strains are Wistar-derived, then between-strain differences unrelated to epilepsy, supposed to be minimal.

Previously we have already made some comparative analyses with the same design for a number of electrophysiological, behavioral and neurochemical parameters [10,14–18]. In particular, we showed that D1DR and D2DR binding densities displayed epilepsy type-specific changes in regions of brain striatal complex, as well as general, epilepsy type-nonspecific alterations [19].

Importantly, quite recently we noticed some pronounced social contact deficits in a rat strain included in our study, the strain of Krushinsky-Molodkina (KM) strain [20], consistently seen in a battery of social preference/social novelty tests [21]. The strain was proposed as a model of Autism Spectrum Disorder (ASD) [20,21], comorbiding with genetic epilepsy in clinical practice [22–24] Due to a need to dissect possible neurophysiological mechanisms, underlying ASD [25–27], we also used our dataset archives to search for putative local dopaminergic effects, correlated with ASD-like behavioral traits in one of our experimental group.

As we mentioned above, in the concept of epilepsy, not only seizures themselves are important, but also their cognitive and emotional consequences. Taking this into account, it seems important to study how the state of dopaminergic brain system changes in the region of the cortex, which is inextricably linked with both the cognitive and emotional components of all goal-directed actions, the prefrontal cortex [28].

The prefrontal cortex is one of the most intriguing regions of the brain even though our understanding of its anatomy and physiology has remarkably evolved over the past decades. The information about this area is still full of mysteries and contradictions, both for anatomy and physiology.

First of all paradoxex, there is still no single generally accepted picture of cortical delineation and nomenclature.

There are two different schemes of cortical parcellation for the rat. First, Paxinos and Watson's [29,30] based on that of Zilles [31], with recent revisions of Zilles scheme [32], and the second one proposed by Swanson [33].

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The change that is difficult not to mention when talking about prefrontal cortex is a delineation and nomenclature. In [34] authors stated that even the term "prefrontal" is incorrect from the point of view of translational science, and should be replaced by the terms "anterior cingulate" and "midcingulate" cortexi now. Those who are interested in criteria for the ACC/MCC division we refer to [35]. Since there is a huge scientific literature using the "old" nomenclature (before 2022) [36], we kept double naming in our manuscript.

Anterior cingulate cortex participates in the coordination of autonomic activities, internal responses to noxios stimulation and emotional states and memories and its' influence can be conditioned in learning paradigms [35]. There is data that area 24b (former Cing1) is involved in emotional vocal expression [35]. Agranular parts of the prefrontal cortex areas (namely 24, 32 and 25) are common both to human and the rat. Therefore, rodent research can provide valuable information about these areas in human [37].

One from the all possible ways to delineate functions of a brain area is study it in normal and pathological conditions. If we are talking about epilepsy there some data about seizures originating from cingulate cortex [38] but less information about changes in the prefrontal cortex that are secondary to generalized epilepsy of any kind.

Thus, in this study we compared D1DR and D2DR density in different areas of the prefrontal cortex of normal rats and rats with different forms of generalized epilepsies, and with comorbiding social contact deficits.

2. Materials and Methods

2.1. Animals

Adult male rats aged 7-9 months and weighing 290-410 g were used. All rats were housed in individual cages under a natural light-dark cycle with free access to water and food.

The study has the same design as previously published (Figure 1) [16–18].

Four experimental groups were formed (Figure 1): healthy Wistar rats from Stolbovaya laboratory animal nursery (n = 5), WAG/Rij rats (descendants of the colony from Radboud University, Nijmegen, the Netherlands) with the "pure" absence-epilepsy (n = 5) and the mixed form of epilepsy (n = 5) and KM rats (the colony bred on the Biological Faculty, Moscow State University, Russia) (n = 5).

All experiments were carried out in accordance with [39] and with the requirements of the Institutional Animal Care Committee.

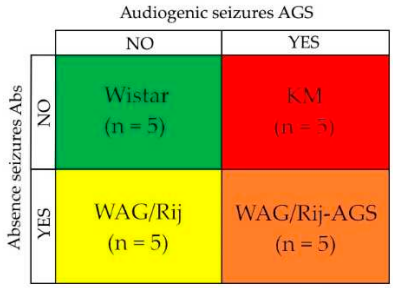

Figure 1. The experimental design implies the comparison of four groups: Wistar control rats (no seizures), KM rats (audiogenic convulsive seizures only), WAG/Rij rats (absence non-convulsive seizures only) and WAG/Rij-AGS rats with mixed form of epilepsy (presence both convulsive and non-convulsive seizures). "YES" means presence and "NO" means absence of the corresponding type of seizures.

2.2. AGS-susceptibility test

The AGS-susceptibility test was performed after three days of habituation to the experimental room, 3 times at a 7-day interval according to [40]. The test was the complex sound (13-85 kHz, 50-60 dB) administered for 90 s. AGS were rated according to Krushinsky's scale [9,41].

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2.3. Decapitation and brain dissection

Rats were decapitated under a deep general anesthesia. Brains were quickly removed and immediately frozen in isopentane kept on dry ice. Coronal slices (14 μm) were cut by cryotome at -18°C at the following levels according to [29] and [34]:

I – AP+2,396-2,513

II – AP+0,173-0,192

III – AP-0,256-0,334

Slices were mounted onto polylysine-coated slides (Menzel-Glaser, Germany). Alternate sections were allocated to slides for total or nonspecific binding or for morphological control. Sections were dried at room temperature overnight and then stored at −20 ◦C until autoradiography.

The Nissl method was used to stain the control slices for morphological control.

2.4. Autoradiography

D1DR and D2DR receptor autoradiography was done as described earlier [42], similar to our previous studies [19,43].

Sections for specific binding were incubated in Tris-buffer containing:

1. For D1DR - 0.2nM [3H]SCH 23390 (specific activity 66.0 Ci/mmol, Amersham) for 90 min at room temperature.
2. For D2DR - 0.4nM [3H]spiperone (specific activity 109.0 Ci/mmol, Amersham) for 60 min at room temperature.

Tris-buffer for non-specific binding contained:

1. For D1DR - 0.2nM [3H]SCH 23390 and 10−7Mcis-flupenthixol.
2. For D2DR - 0.4nM [3H]spiperone, 10−5M haloperidol and 10−5M ketanserin.

After incubation, the slides were drained, washed two times for 5 min in buffer at +4 ◦C and briefly dipped two times into distilled water (+4 ◦C). Sections were dried at room temperature overnight and exposed to a tritium-sensitive film (3H Hyperfilm®, Amersham) at −20 ◦C together with Amersham 3H Microscale Autoradiography Standards®, within 4 weeks for D1DR and 3 weeks - for D2DR. Then all films were developed, digitized and analyzed with ImageJ (https://imagej.net/ij/) [44].

2.5. Measurements

The scanned images of autoradiography films had been contrasted digitally to the level of clear distinction of prefrontal regions and processed with the effect of a thermal imager (https://www.imgonline.com.ua/add-effect-thermal-imager.php). The brain structures were identified according to [29] and [34]. Measurements were carried out in the medial and lateral aspects of the prefrontal cortex (Table 1), the parcellation is shown on Figure 2. Recognizing the difficulty of transitioning from the old nomenclature to the new one, we decided to use both versions as a temporary measure to get used to the changes.

Table 1. List of studied areas of prefrontal cortex with abbreviations and the comparison of the nomenclature used in literature ([29,30]. Modified according to [34]).

6th edition, 2007[30]	**7th edition, 2014[29]**
Medial prefrontal cortex	**Medial frontal cortex, anterior cingulate cortex (ACC)**
Infralimbic cortex IL	A25
Prelimbic cortex PL	A24a
Primary cingulate cortex Cing1	A24b
Secondary cingulate cortex Cing2	A24a

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	Homologue of area 33 A33
	Midcingulate cortex (MCC)
Primary midcingulate cortex mCing1	A24b′
Secondary midcingulate cortex mCing2	A24a′
Lateral prefrontal cortex	**Insular cortex**
Granular insular cortex GI	Granular insular cortex GI
Dysgranular insular cortex DI	Dysgranular insular cortex DI
Dorsal agranular insular cortex AID	Dorsal agranular insular cortex AID
Ventral agranular insular cortex AIV	Ventral agranular insular cortex AIV
Posterior agranular insular cortex AIP	Posterior agranular insular cortex AIP

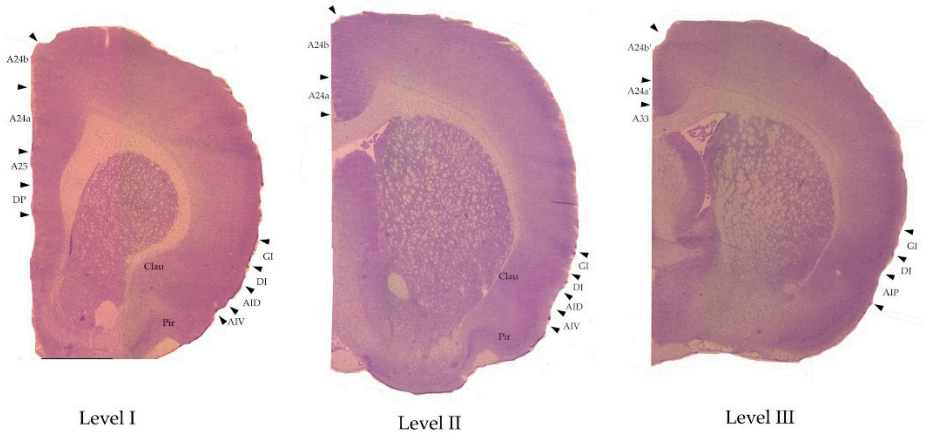

Figure 2. The regions of interest marked on examples of the Nissle-stained brain slices. that were made for the morphological control. The anatomical levels are AP: +2,396-2,513 (level I), +0,173-0,192 (level II) and -0,256-0,295 (level III) according to [29] and [34]. For abbreviations see Table 1.

The obtained values of optical densities were converted into pmol/g of tissue by using the microscale standards (see Section 2.4).

2.6. Statistical analysis

Statistical analysis was performed with Statistica 10.0 (TIBCO Software Inc., Pao Alto, CA, USA). Values are shown as means ± SEM.

Analysis of D1DR and D2DR binding densities in a defined cortical region was performed for each anatomical level separately, using ANOVA GLM (General Linear Models), similarly as described early [18,19]. Briefly, to account for variations caused by non-homogeneities of the films, the local background levels were taken as continuous GLM predictors, and the films' ID were taken as a categorical predictor; their effects are not reported below. Anatomical levels were analyzed separately; two brain hemispheres were taken as within-subjects factors, so a repeated measures analysis was used. ANOVA GLM for regional data was run with the epilepsy type (AGS and/or AbS, 2 × 2 design) as two between-subjects factors (Figure 1). This analysis provided information about the general effect of AGS/AbS susceptibilities, as previously done [18,19]. The putative effects of AGS were checked by comparing the pooled group of KM and WAG/Rij-AGS rats with the pooled group of AGS-unsusceptible rats (i.e., WS and WAG/Rij). The effects of AbS' proneness were estimated by comparing the pooled groups of WAG/Rij and WAG/Rij-AGS rats with the pooled groups of WS and KM rats. The general effect of epilepsy ("factor EPILEPSY") was assessed by comparing normal Wistar rats with the pooled data of the three groups of epileptic rats (i.e., KM, WAG/Rij, and WAG/Rij-AGS). The general effect of ASD-like phenotype ("factor AUT") was assessed by comparing

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socially normotypic group (pooled Wistar, WAG/Rij and WAG/Rij-AGS rats) with the data of socially-deficient rats (i.e. KM). For these cases, general ANOVAs were followed by post-hoc tests (Unequal N HSD test). The minimal level of significance was set at p = 0.05.

3. Results

3.1. D1-DR binding density

General distribution of D1DR within the prefrontal territories studied is in line with the previously published [28,45]. It is exemplified on Figure 3. Briefly, the lateral regions of prefrontal (insular) cortex displayed a moderate binding to D1DR, with the maximal values seen in claustrum (Figure 3). The rostro-caudal gradients were mild for D1DR binding densities, in a contrast to the profile previously reported H3 histamine receptor binding [18]. The rat groups differed in local levels of D1DR binding densities, the most of variations were explained by proneness to epilepsy, or to social behavioral phenotype. Effects of specific epilepsy type (i.e., audiogenic and/or absence) were estimated by ANOVA GLM, as described in below (Subchapters 4.1.1); interaction effects were further studied in post-hoc tests (Subchapters 3.3 and 3.4).

Absence-epileptic WAG/Rij rats demonstrated a decreased D1DR binding in A24a/prelimbic region of prefrontal cortex (Figure 3A). A tendency to AbS-related elevation was observed in the granular insular cortex (Figure 3B).

The convulsive form of epilepsy (AGS), was associated with a decrease of D1DR binding in rostral divisions of lateral prefrontal cortex: disgranular insular (DI), agranular insular dorsal (AID) and ventral (AIV) (Figure 3B).

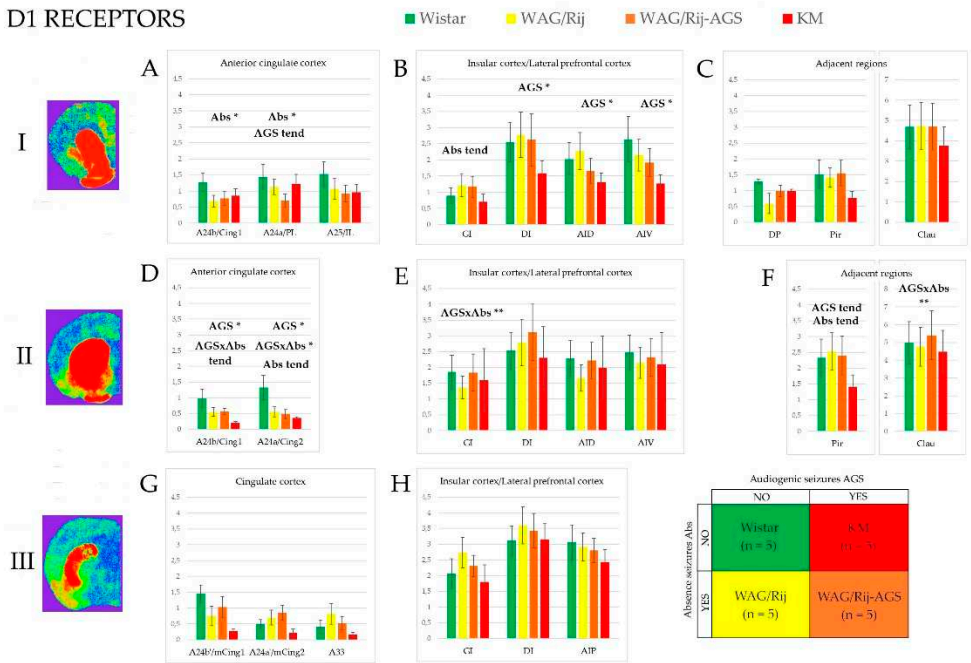

Figure 3. Comparison of the D1DR regional binding densities in the prefrontal cortex and some adjacent regions between the pooled groups (see Materials and Methods, Section 2.6). The values are given as mean ± SEM, in pmol/g of tissue. Rows of charts corresponds to anatomical levels which are AP: +2,396-2,513 (level I – first row of charts top down), +0,173-0,192 (level II – the second row) and -0,256-0,295 (level III – the last row) according to [29] and [34]. Next to level numbers are examples of brain slices from the corresponding levels, processed with a thermal imager. Graphs A, D and G show D1DR density for the anterior cingulate and midcingulate cortices (former medial prefrontal cortex), graphs B, E and H – insular cortex (former lateral prefrontal cortex) and graphs C and F – D1DR density in some regions adjacent to prefrontal cortex. For anatomical abbreviations (x axis) see Materials and Methods (Section 2.5, Table 1). Differences are denoted by * for p≤0,05, ** - for p≤0,01 and

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"tend" for tendency (0,05<p<0,10). The "AGS" sign marks the effect of the AGS presence and "abs" – the absence epilepsy.

3.2. *D2/3-DR binding density*

The distribution of local binding densities to D2DR is shown on Figure 4. In general, cortical pattern of D2DR binding agrees with the scientific literature [28,45]. In brief, the lateral prefrontal cortex, as well as claustrum, revealed noticeable levels of D2DR binding densities, with clear typical lamination pattern (Figure 4)

Absence-epileptic WAG/Rij rats demonstrated a decreased D2DR binding in A24a/prelimbic region of prefrontal cortex (Figure 4A), dysgranular insular cortex (Figure 4B), agranular insular cortex (Figure 4E) and dorsal peduncle (Figure 4C).

The convulsive form of epilepsy (AGS) was associated with an increased D2DR binding density in dorsal agranular insular cortex (Figure 4B), mostly on expense of KM rats (red bars on Figures 3 and 4)

Interaction of AGS*AbS factors were seen in a number of areas studied (Figure 4); such cases were further analyzed in post-hoc tests (see Sections 3.3 and 3.4).

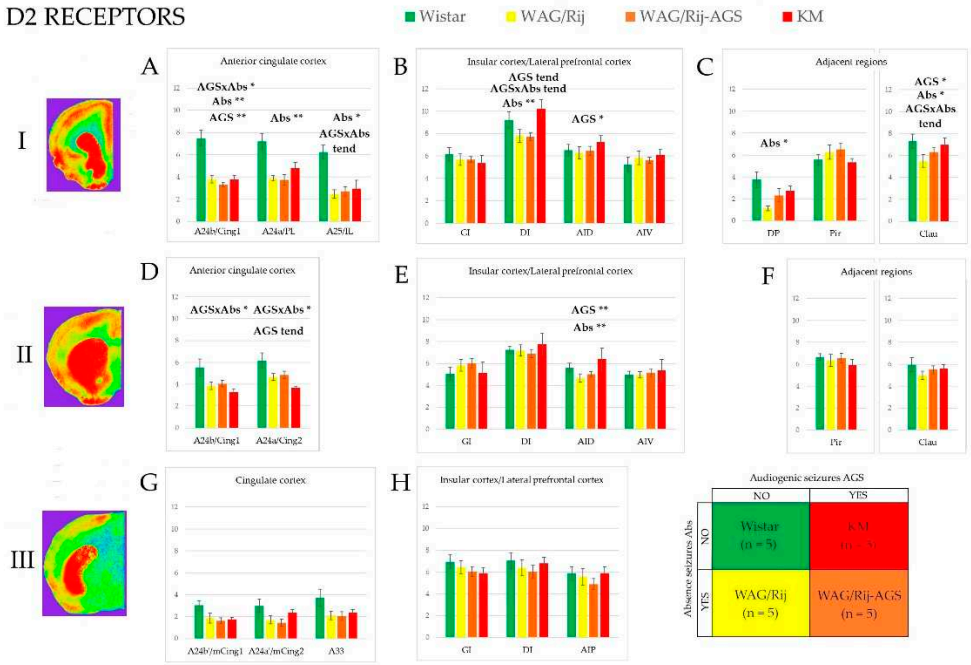

Figure 4. Comparison of the D2DR regional binding densities in the prefrontal cortex and some adjacent regions between the pooled groups (see Materials and Methods, Section 2.6). The values are given as mean ± SEM, in pmol/g of tissue. Lines of charts corresponds to anatomical levels which are AP: +2,396-2,513 (level I – first line of charts top down), +0,173-0,192 (level II – the second line) and -0,256-0,295 (level III – the last line) according to [29] and [34]. Next to level numbers are examples of brain slices from the corresponding levels after processing with the effect of a thermal imager. Graphs A, D and G show D2DR density for the anterior cingulate and midcingulate cortices (former medial prefrontal cortex), graphs B, E and H – insular cortex (former lateral prefrontal cortex) and graphs C and F – D2DR density in some regions adjacent to prefrontal cortex. For anatomical abbreviations (x axis) see Materials and Methods (Section 2.5, Table 1). Differences are denoted by * for p≤0,05, ** - for p≤0,01 and "tend" for tendency (0,05<p<0,10). The "AGS" sign marks the effect of the AGS presence and "abs" – the absence epilepsy.

3.3. *Effect of epilepsies*

In the present study we saw general impacts of epilepsies on D1DR and D2DR systems, similarly as it was recently reported for H3 histamine autoreceptors [18]. In anterior cingulate cortex (both primary and secondary), the three groups of epileptic rats (KM, WAG/Rij and WAG/Rij-AGS) didn't

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majorly differed from each other, but displayed a concordant decrease in local binding densities (Figure 3A,D). Being pooled to form an "epileptic" group (white bars on Figure 5) and compared to the control mother strain (Wistar rats, green bars on Figure 5), the rats with genetic generalized epilepsies demonstrated significantly decreased in D1DR, D2DR binding densities, as measured in the primary and secondary cingulate cortices, prelimbic and infralimbic areas (regions A24a, A24b, A25 in translational terminology [29,34]).

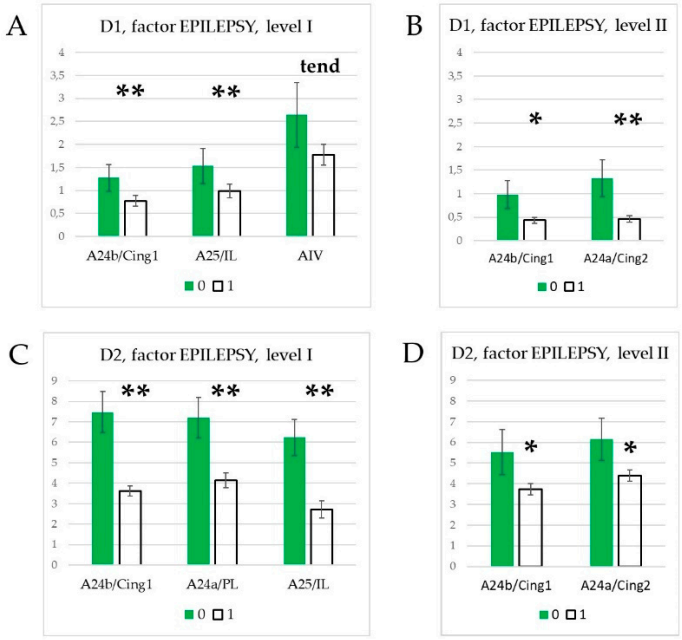

Figure 5. Effects of genetic generalized epilepsy in D1DR and D2DR binding densities. Graphs A and B show structures for which the D1DR density was significantly different for rats with epilepsy on level I and II respectively. Graphs C and D show structures for which the D2DR density was significantly different for rats with epilepsy on level I and II respectively. "0" = no epilepsy, "1" – presence of epilepsy. For anatomical abbreviations (x axis) see Materials and Methods (Section 3. 5, Table 1). Differences are denoted by * for p≤0,05, ** - for p≤0,01 and "tend" for tendency (0,05<p<0,10).

Thus, the effects of generalized epilepsies in the present study were seen as fainted binding (Figure 6), specifically localized in the regions of medial/anterior cingulate prefrontal cortex, and found both for D1DR and D2DR system. To check whether such a concordant decrease might be related to a secondary factor (like mechanical qualities of brain tissue), we run a correlation analysis of the whole dataset (Table 2A,B). The results show, that the D1DR and D2DR subsets were largely independent, both for in control and epileptic rats' subsets.

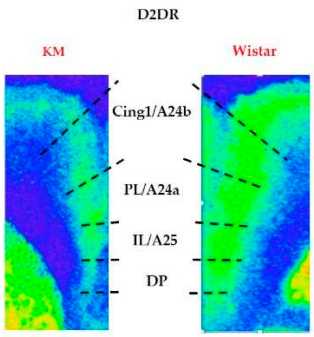

Figure 6. An example of D2DR binding densities' regional distribution in medial prefrontal/anterior cingulate cortex of an epileptic (left) and normal (right) rat brains.

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Table 2A. Correlations of local D1DR and D2DR binding densities in structures of medial and lateral aspects of prefrontal cortex in non-epileptic (Wistar) rats

Spearman rank order coefficients are given for significant correlations; non-significant correlations not shown.

		D1DR A24b	D1DR A24a	D1DR A25	D1DR DP	D1DR GI	D1DR DI	D1DR AIV	D1DR AID	D1DR CIau	D2DR A24b	D2DR A24a	D2DR A25	D2DR DP	D2DR GI	D2DR DI	D2DR AIV	D2DR AID	D2DR CIau
medial	D1DR Cing1/A24b		0,84	0,94	0,95		0,91	0,86	0,96	0,80		0,64							
	D1DR PL/A24a	0,84		0,87	0,88		0,86	0,83	0,86	0,87	0,68	0,83							
	D1DR IL/A25	0,94	0,87		0,94		0,94	0,85	0,92	0,88		0,71	0,66						
	D1DR DP	0,95	0,88	0,94			0,90	0,84	0,96	0,90		0,72							
lateral	D1DR GI											0,84	0,73						
	D1DR DI	0,91	0,86	0,94	0,90			0,98	0,94	0,84		0,73	0,73						
	D1DR AID	0,86	0,83	0,85	0,84		0,98		0,94	0,80		0,67	0,67						
	D1DR AIV	0,96	0,86	0,92	0,96		0,96	0,94		0,84		0,69	0,69						
	D1DR Clau	0,80	0,87	0,88	0,90		0,84	0,80	0,84		0,68	0,71	0,63						
medial	D2DR Cing1/A24b		0,68							0,68		0,68	0,75						
	D2DR PL/A24a	0,64	0,83	0,71	0,72	0,84	0,73	0,67	0,69	0,71	0,68		0,83						
	D2DR IL/A25			0,66		0,73	0,73	0,67	0,69	0,63	0,75	0,83							
	D2DR DP														0,87	0,90	0,72	0,70	0,65
lateral	D2DR GI													0,87		0,95	0,82	0,81	0,76
	D2DR DI													0,90	0,95		0,76	0,82	0,77
	D2DR AID													0,72	0,82	0,76		0,92	0,90
	D2DR AIV													0,70	0,81	0,82	0,92		0,96
	D2DR Clau													0,65	0,76	0,77	0,90	0,96	

Table 2B. Correlations of local D1DRDR and D2DRDR binding densities in prefrontal structures of medial and lateral aspects of prefrontal cortex in epileptic rats

Spearman rank order coefficients are given for significant correlations; non-significant correlations not shown.

		D1DR A24b	D1DR A24a	D1DR A25	D1DR DP	D1DR GI	D1DR DI	D1DR AIV	D1DR AID	D1DR CIau	D2DR A24b	D2DR A24a	D2DR A25	D2DR DP	D2DR GI	D2DR DI	D2DR AIV	D2DR AID	D2DR CIau
medial	D1DR Cing1/A24b		0,84	0,87	0,82	0,56	0,75	0,72	0,69	0,84					0,40				0,42
	D1DR PL/A24a	0,84		0,83	0,74	0,72	0,75	0,78	0,73	0,83									
	D1DR IL/A25	0,87	0,83		0,81	0,57	0,74	0,74	0,73	0,82		0,40			0,41				0,36
	D1DR DP	0,82	0,74	0,81		0,47	0,68	0,69	0,71	0,78									0,40
lateral	D1DR GI	0,56	0,72	0,57	0,47		0,75	0,75	0,74	0,74									
	D1DR DI	0,75	0,75	0,74	0,68	0,75		0,96	0,95	0,93					0,46				
	D1DR AID	0,72	0,78	0,74	0,69	0,75	0,96		0,97	0,91					0,46				
	D1DR AIV	0,69	0,73	0,73	0,71	0,74	0,95	0,97		0,92					0,41				
	D1DR Clau	0,84	0,83	0,82	0,78	0,74	0,93	0,91	0,92						0,42				
medial	D2DR Cing1/A24b														0,48	0,47	0,51	0,72	0,47
	D2DR PL/A24a			0,40									0,50	0,49		0,46	0,48		0,39
	D2DR IL/A25											0,50		0,64					
	D2DR DP											0,49	0,64						
lateral	D2DR GI	0,40		0,41			0,46	0,46	0,41	0,42	0,48					0,55	0,52	0,63	0,61
	D2DR DI										0,47	0,46			0,55		0,75	0,79	0,93
	D2DR AID										0,51	0,48			0,52	0,75		0,72	0,71
	D2DR AIV										0,72				0,63	0,79	0,72		0,78
	D2DR Clau	0,42		0,36	0,40						0,47	0,39			0,61	0,93	0,71	0,78	

3.4. Effect of social phenotype.

A post-hoc comparisons were made to assess the difference between the three groups of "socially normotypic" rats (i.e., Wistar, WAG/Rij and WAG/Rij-AGS) and the rats with ASD-like phenotype (i.e., KM rats). The analysis revealed a difference between KM rats and three other rat groups pooled to form "socially normotypic" cohort. Namely, the regions affected were mainly structures of lateral prefrontal cortex: dysgranular and dorsal agranular insular cortex, together with neighboring claustrum and piriform cortex (Figures 7 and 8). The effects for D1DR were opposite to those seen for D2DR. The pooled group of normotypic rats demonstrated higher binding densities to D1DR in dysgranular insular cortex and two adjacent areas (claustrum and piriform cortex) as compared to socially deficient KM rats (Figure 7A,B). In a contrast, the group of "normotypic" rats was lower in local binding to D2DR, as measured in dorsal agranular insular cortex and dysgranular insular cortex (Figure 7 C,D).

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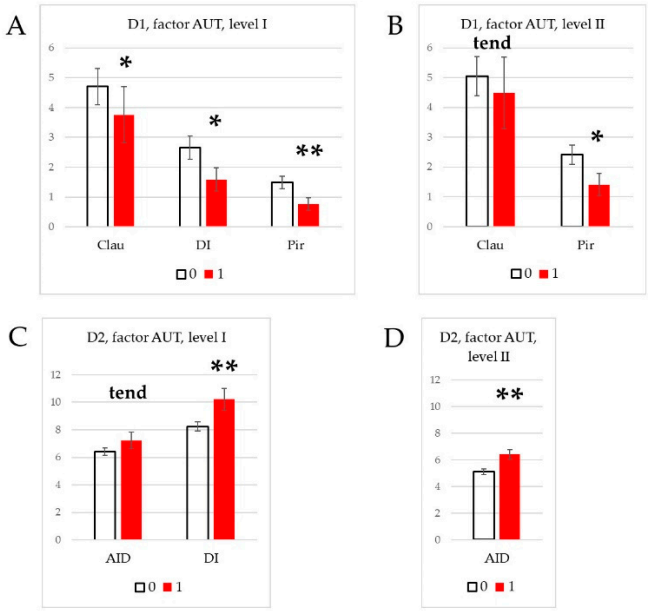

Figure 7. Impacts of ASD-like phenotype in D1DR and D2DR binding densities. Graphs A and B show structures for which the D1DR density significantly differed between rats with ASD-like phenotype (red bars) and normotypic rats (pooled group, white bars), on anatomical level I and II respectively. Graphs C and D show structures for which the D2DR density significantly differed between the rats with ASD-like phenotype (red bars) and normotypic ones (white bars), on anatomical level I and II respectively. "0" = normotypical social traits, "1" – ASD-like phenotype. For anatomical abbreviations (x axis) see Materials and Methods (Section 2. 5, Table 1). Differences are denoted by * for p≤0,05, ** - for p≤0,01 and "tend" for tendency (0,05<p<0,10).

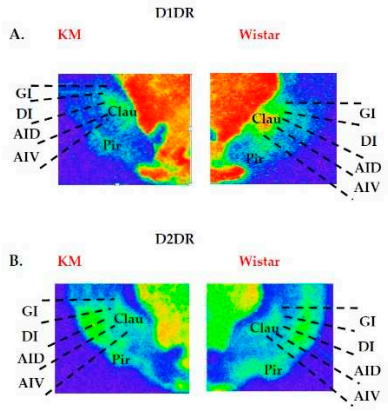

Figure 8. An example of D1DR (A) and D2DR (B) regional binding densities' distribution in medial prefrontal/anterior cingulate cortex of an "autistic" (left) and "normotypic" (right) rat brains.

3.3. Correlations between local binding densities to D1DR and D2DR within the prefrontal regions

The subsets of local values for D1DR and D2DR binding densities were studied by Spearman rank order correlations, to see putative functional interrelations of prefrontal regions, and outline possible artifacts. The Rs values (Spearman coefficients) for normal and epileptic groups are given in Table 2A,B, respectively.

The results demonstrated majorly independent local patterns seen for D1DR and D2DR local bindings (low left quadrants of Table 2A,B).

 doi:10.20944/preprints202309.1353.v1

The D1DR data were seen to be closely interrelated, since the majority of regions revealed significant positive correlations (upper left quadrants of Table 2A,B). It means that a local value for D1DR in a region of prefrontal cortex might serve as a predictor for any other D1DR local binding density; remarkably the medial and lateral aspects of prefrontal cortex were well correlated.

The correlation pattern seen within the D2DR subset (low right quadrants of Table 2A,B) was more complex and demonstrated a lower number of links; it split to medial and lateral subregions. The D2DR binding density patterns largely correlated within the lateral aspects of prefrontal cortex, but this was less clear for the medial ones.

4. Discussion

4.1. Effects of specific epilepsy types.

The effects of particular epilepsy types were moderate in this study, in a contrast to the previous ones [16,18,19]. The prefrontal regions studied are not directly involved in generation of seizures of absence and/or audiogenic epilepsies. Rather, the changes reported above might be attributed to behavioral comorbidities of epilepsies, found in a significant part of patients and affecting their quality of life [22,23].

Observed rostro-caudal D1DR and D2DR densities' gradients, as well as the fact that significant differents were found only in the anterior regions are in line with the known anterior and posterior divisions of rodent and human insular cortex [46].

4.2. Effects of generalized epilepsies

We recently described a prominent decrease in local binding densities to H3 histamine receptors, seen in the same set of experimental animals. Namely, structures of medial and lateral aspects of prefrontal cortex demonstrated a widespread significant fainting in local binding densities to H3 histamine receptor: primary cingulate and secondary midcingulate areas, dorsal agranular cortex, granular and dysgranular cortexi [18].

Here, we saw a more localized decrease in brain dopaminergic receptor bindings: the effects of "epilepsy" on binding to D1DR and D2DR were predominantly seen in the medial areas (Figure 5). Correlation analysis showed that the local decreases in binding to D1DR were not depended on those seen for D2DR (see SubChapter 4.3 and Table 1). So, we might hypothesize that the observed 20-50% lowering in anterior cingulate binding to D1DR and D2DR does not stem from a single process. Taking into a consideration the wider pattern of receptor binding decrease seen for H3 histamine receptors of the same animals [18], it is possible to expect an epilepsy-related multi-staged degeneration of brain aminergic systems. Also, it was shown that H3-reverse agonist significantly increased extracellular dopamine concentration in the rat prefrontal cortex [47].

Neuroinflammation would be a putative candidate for a triggering such a multi-step process resulting in independent diffuse lowering of aminergic tone.

4.3. Effects of social phenotype

It was shown that rats with any form of epilepsy, like humans, show various comorbid conditions which makes them both more valid and valuable and, at the same time, more difficult in terms of interpreting the results.

Comparisons of KM rats (with ASD-like phenotype) versus the pooled cohort of the three groups (i.e., Wistar, WAG/Rij, WAG/Rij-AGS) of rats without social deficits [48], resulted in a specific pattern of D1DR, D2DR bindings affected (Figures 7 and 8). Namely, the regions of insular cortex, together with neighboring claustrum were altered in KM rats as compared to socially "normotypic" group (Figure 7). Interestingly, this putatively ASD-related pattern was specifically localized in the lateral aspects of prefrontal cortex. Insular cortex is a place of convergence for interoceptory sensory inflow and emotional processing in limbic circuits [46,49]. In humans, the insular cortex is referred as a structure responsible for "embodied emotions [49]. Although any conclusive remarks on the insular

dopaminergic effects on ASD-like phenotype would be immature at this stage, future researches are warranted to clarify the matter.

It has to be mentioned that the results reported above does not produce a straightforward conclusion on desired neurochemical correlates of social deficits, since KM strain is also characterized by arterial hypertension [50,51]. Dopaminergic dysfunctions of the insular regions and claustrum might be also related to that. In literature, more caudal insular region (namely, posterior agranular insular cortex) is referred as responsible for arterial pressure in rats [52], which was not marked by altered binding densities to D1DR or D2DR in our study (Figures 3H and 4H). Taking in mind that in general, the granular and dysgranular insular cortices are considered as the primary interoceptive cortex [53], and agranular regions of insular cortex are referred as association sensory cortex [53–55], we might hypothesize that interception is rather dysfunctional in KM rats. It doesn't contradict with a putative involvement of insular dopaminergic system in pathophysiology of social deficits in KM rats, since interoception is aberrant in ASD patients [56]. In parallel, it is known that ASD patients suffer from a significantly higher number of somatic medical issues [57], as compared to the general populations. So, the arterial hypertension might be one of the somatic dysfunction comorbiding with ASD-like phenotypes. A growing incidence of ASD diagnosis in clinical practice warrants future experimentations to clarify a role of dopaminergic innervations of insular cortex in behavioral deficits. KM rats might serve as an experimental animal model for this.

A clear limitation of this study is an absence of female rats in this study. However, the results obtained should be to taken rather to map the cortical regions enrolled in pathological processes related to epilepsy and its psychiatric comorbidities. Further research is needed to specify the particular roles of each receptor systems in these regions.

Author Contributions: Conceptualization, I.S.M. and G.v.L.; methodology, L.M.B.; data curation, I.S.M.; investigation, L.M.B. and I.S.M.; writing—original draft preparation, L.M.B.; writing—review and editing, I.S.M. and G.v.L; visualization, I.S.M.; project administration, G.v.L. All authors have read and agreed to the published version of the manuscript.

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

References

1. Holz, R.W.; Fisher S.K. Chapter 12. Synaptic transmission and cellular signailng: An overview. In *Basic neurochemistry: Principles of molecular, cellular and medical neurobiology*, 8th ed.; Brady, S.T., Siegel G.J., Eds. Elsevier Academic Press, 2012, pp. 235-257.
2. Cumming, P. *Imaging dopamine*. Cambridge University Press, 2009.
3. *Dopamine receptor technologies*. Tiberi, M., ed. Humana Press, Springer Science+Business Media New York, 2015.
4. George, S.R.; Kern, A.; Smith, R.G.; Franco R. Chapter 8 – Dopamine receptor heteromeric complexes and their emerging functions. *Prog Brain Res*, **2014**, 211, pp. 183-200. doi: 10.1016/B978-0-444-63425-2.00008-8.
5. Akyuz, E.; Polat, A.K.; Eroglu, E.; Kullu, I.; Angelopoulou, E.; Paudel, Y.N. Revisiting the role of neurotransmitters in epilepsy: An updated review. *Life Sci*., **2021**, 265, 118826. doi: 10.1016/j.lfs.2020.118826.
6. Coenen, A.M.; van Luijtelaar, E.L. Genetic animal models for absence epilepsy: a review of the WAG/Rij strain of rats. *Behav Genet*. **2003**, 33(6), 635-655. doi: 10.1023/a:1026179013847.
7. Van Luijtelaar, G.; Zobeiri, M. Progress and outlooks in a genetic absence epilepsy model (WAG/Rij). *Curr Med Chem*, **2014**, 21(6), 704-21. doi: 10.2174/09298673206613119152913.
8. Van Luijtelaar, G.; van Oijen, G. Establishing Drug Effects on Electrocorticographic Activity in a Genetic Absence Epilepsy Model: Advances and Pitfalls. *Front Pharmacol*. **2020**, 11, 395. doi: 10.3389/fphar.2020.00395.
9. Kuznetsova, G.D. Audiogenic seizures in rats of different genetic strains. *Zh Vyss. Nervn Dejat* **1998**, 48, 143–152. (In Russian)
10. Midzyanovskaya, I.S.; Kuznetsova, G.D.; Vinogradova, L.V.; Shatskova, A.B.; Coenen, A.M.; van Luijtelaar, G. Mixed forms of epilepsy in a subpopulation of WAG/Rij rats. *Epilepsy Behav*. **2004**, 5(5), 655-661. doi: 10.1016/j.yebeh.2004.06.021.

11. Poletaeva, I.I.; Surina, N.M.; Kostina, Z.A.; Perepelkina, O.V.; Fedotova, I.B. The Krushinsky-Molodkina rat strain: The study of audiogenic epilepsy for 65 years. *Epilepsy Behav*. **2017**, 71(Pt B), 130-141. doi: 10.1016/j.yebeh.2015.04.072.
12. Fedotova, I.B.; Surina, N.M.; Nikolaev, G.M.; Revishchin, A.V.; Poletaeva, I.I. Rodent brain pathology, audiogenic epilepsy. *Biomedicines*, **2021**, 9 (11), 1641. doi: 10.3390/biomedicines9111641
13. Garbuz, D.G.; Davletshin, A.A.; Litvinova, S.A.; Fedotova, I.B.; Surina, N.M.; Poletaeva, I.I. Rodent Models of Audiogenic Epilepsy: Genetic Aspects, Advantages, Current Problems and Perspectives. *Biomedicines*. **2022**, 10(11), 2934. doi: 10.3390/biomedicines10112934.
14. Midzyanovskaya, I.S.; Shatskova, A.B.; Sarkisova, K.Y.; van Luijtelaar, G.; Tuomisto, L.; Kuznetsova G.D. Convulsive and nonconvulsive epilepsy in rats: effects on behavioral response to novelty stress. *Epilepsy Behav*. **2005**, 6(4), 543-551. doi: 10.1016/j.yebeh.2005.03.005.
15. Midzyanovskaya, I.S.; Kuznetsova, G.D.; van Luijtelaar, E.L.; van Rijn, C.M.; Tuomisto, L.; Macdonald E. The brain 5HTergic response to an acute sound stress in rats with generalized (absence and audiogenic) epilepsy. *Brain Res Bull*. **2006**, 69(6), 631-638. doi: 10.1016/j.brainresbull.2006.03.008.
16. Midzyanovskaya, I.S.; Birioukova, L.M.; Shatskova, A.B.; van Luijtelaar G.; Tuomisto L.M. H1 histamine receptor densities are increased in brain regions of rats with genetically generalized epilepsies. *Epilepsy Res*, **2016**, 127, 135-140.
17. Midzyanovskaya, I.S.; Petrenko, T.E.; Birioukova, L.M.; Tuomisto, L.M. Reduced H3 histamine receptor binding densities in the upper layers of motor cortex in rats prone to audiogenic convulsive seizures. *Epilepsy Res*., **2021**, 170, 106543. doi: 10.1016/j.eplepsyres.2020.106543.
18. Midzyanovskaya, I.S.; Birioukova, L.M.; Storvik, M.; van Luijtelaar, G.; Tuomisto, L.M. The prefrontal cortex shows widespread decrease in H3 histamine receptor binding densities in rats with genetic generalized epilepsies. *Epilepsy Res*. **2022**, 182, 106921. doi: 10.1016/j.eplepsyres.2022.106921.
19. Tsyba, E.T.; Midzyanovskaya, I.S.; Birioukova, L.M.; Tuomisto, L.M.; van Luijtelaar, G.; Abbasova K.R. Striatal patchwork of D1-like and D2-like receptors binding densities in rats with genetic audiogenic and absence epilepsies. *Diagnostics (Basel)*, **2023**, 13(4), 587. doi: 10.3390/diagnostics13040587
20. Rebik, A.A.; Riga, V.D.; Smirnov, K.S.; Sysoeva, O.V.; Midzyanovskaya, I.S. Social Behavioral Deficits in Krushinsky-Molodkina Rats, an Animal Model of Audiogenic Epilepsy. *J Pers Med*, **2022**, 12(12), 2062. doi: 10.3390/jpm12122062.
21. Rebik, A.A.; Broshevitskaya, N.; Kuzhuget, S.; Aleksandrov P.; Abbasova K.; Zaichenko, M.; Midzyanovskaya, I.S. Audiogenic Seizures and Social Deficits: No Aggravation Found in KM Rats. *Biomedicines*, **2023**, *in press*.
22. Besag, F. Epilepsy in Patients with Autism: Links, Risks and Treatment Challenges. *Neuropsychiatr. Dis. Treat.* **2017**, 14, 1–10. https://doi.org/10.2147/NDT.S120509.
23. Brooks-Kayal, A. Epilepsy and autism spectrum disorders: Are there common developmental mechanisms? *Brain Dev*. **2010**, 32, 731–738. https://doi.org/10.1016/j.braindev.2010.04.010.
24. Bozzi, Y.; Provenzano, G.; Casarosa, S. Neurobiological bases of autism-epilepsy comorbidity: a focus on excitation/inhibition imbalance. *Eur J Neurosci*. **2018**, 47(6), 534-548. doi: 10.1111/ejn.13595.
25. Silverman, J.L.; Thurm, A.; Ethridge, S.B.; Soller, M.M.; Petkova, S.P.; Abel, T.; Bauman, M.D.; Brodkin, E.S.; Harony-Nicolas, H.; Wöhr, M.; Halladay, A. Reconsidering animal models used to study autism spectrum disorder: Current state and optimizing future. *Genes Brain Behav*, **2022**, 21(5), e12803. https://doi.org/10.1111/gbb.12803
26. Sierra-Arregui, T.; Llorente, J.; Giménez Minguez, P.; Tønnesen, J.; Peñagarikano, O. Neurobiological Mechanisms of Autism Spectrum Disorder and Epilepsy, Insights from Animal Models. *Neuroscience*. **2020**, 445, 69-82. doi: 10.1016/j.neuroscience.2020.02.043.
27. Haratizadeh, S.; Parvan, M.; Mohammadi, S.; Shabani, M.; Nozari, M. An overview of modeling and behavioral assessment of autism in the rodent. *Int J Dev Neurosci*. **2021**, 81(3), 221-228. doi: 10.1002/jdn.10096.
28. Fuster, J.M. *The prefrontal cortex*, 5th ed.; Elsevier Academic Press, 2015.
29. Paxinos, G.; Watson, C. *The rat brain in stereotaxic coordinates*, 7th ed.; Elsevier Academic Press: San Diego, 2014.
30. Paxinos, G.; Watson, C. *The rat brain in stereotaxic coordinates*, 6th ed.; Elsevier Academic Press, San Diego, 2007.
31. Zilles, K. *The cortex of the rat: A stereotaxic atlas*. Springer-Verlag, Berlin, 1985.

32. Vogt, B.A.; Paxinos, G. Cytoarchitecture of mouse and rat cingulate cortex with human homologies. *Brain Struct Funct* **2014**, 219, 185-192.
33. Swanson, L.W. Brain maps 4.0 – Structure of the rat brain: An open access atlas with global nervous system nomenclature ontology and flatmaps. *J Comp Neurol* **2018**, 526, 935-943.
34. Paxinos, G.; Kassem, M.S.; Kirkcaldie M.; Carrive P. *Chemoarchitectonic atlas of the rat brain*, 3rd ed.; Elsevier Academic Press, 2022
35. Vogt, B.A. Cingulate cortex and pain architecture. In *The rat nervous system*, 4th ed. Paxinos, G., ed. Elsevier Academic Press: San Diego, 2015; pp. 575-600.
36. Laubach, M.; Amarante, L.M.; Swanson, K.; White, S.R. What, If Anything, Is Rodent Prefrontal Cortex? *eNeuro*. **2018**, 25, 5(5):ENEURO.0315-18.2018. doi: 10.1523/ENEURO.0315-18.2018.
37. Preuss, T.M.; Wise S.P. Evolution of prefrontal cortex. *Neuropsychopharmacology*, **2022**, 47(1), 3-19. doi: 10.1038/s41386-021-01076-5
38. Inoyama, K.; Devinsky, O. Chapter 19 - Cingulate seizures and recent treatment strategies. In *Cingulate Cortex*, 1st ed.; Handbook of Clinical Neurology, Vol.166. Vogt, B.A., Ed. Elsevier, 2019, pp. 341-353.
39. Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes Text with EEA relevance. Official Journal of the European Union. Available online: http://data.europa.eu/eli/dir/2010/63/oj (accessed on 20 December 2022).
40. Morimoto, K.; Fahnestock, M.; Racine, R. J. Kindling and Status Epilepticus Models of Epilepsy: Rewiring the Brain. *Prog. Neurobiol*. **2004**, 73 (1), 1–60. https://doi.org/10.1016/j.pneurobio.2004.03.009.
41. Vinogradova, L.V. Audiogenic kindling and secondary subcortico-cortical epileptogenesis: Behavioral correlates and electrographic features. *Epilepsy Behav*. **2017**, 71 (Pt B), 142–153. https://doi.org/10.1016/j.yebeh.2015.06.014.
42. Blunt, S.B.; Jenner, P.; Marsden, C.D. Autoradiographic study of striatal D1- and D2-like dopamine receptors in 6-OHDA lesioned rats receiving foetal ventral mesencephalic grafts and chronic treatment with L-DOPA and carbidopa. *Brain Res*. **1992**, 582, (2), 299–311.
43. Birioukova, L.M.; Midzyanovskaya, I.S.; Lensu, S.; Tuomisto, L.; van Luijtelaar, G. Distribution of D1-like and D2-like dopamine receptors in the brain of genetic epileptic WAG/Rij rats. *Epilepsy Res*. **2005**, 63(2-3), 89-96. doi: 10.1016/j.eplepsyres.2004.12.001.
44. Schneider, C. A.; Rasband, W. S.; Eliceiri, K. W. NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, **2012**, 9(7), 671–675. doi:10.1038/nmeth.2089
45. Santana, N.; Artigas, F. Laminar and Cellular Distribution of Monoamine Receptors in Rat Medial Prefrontal Cortex. *Front Neuroanat*. **2017**, 11, 87. doi: 10.3389/fnana.2017.00087.
46. McGregor, M.S.; LaLumiere, R.T. Still a "hidden island"? The rodent insular cortex in drug seeking, reward, and risk. *Neurosci Biobehav Rev*. **2023**, 29, 153:105334. doi: 10.1016/j.neubiorev.2023.105334.
47. Flik, G.; Folgering, J.H.; Cremers, T.I.; Westerink, B.H.; Dremencov E. Interaction Between Brain Histamine and Serotonin, Norepinephrine, and Dopamine Systems: In Vivo Microdialysis and Electrophysiology Study. *J Mol Neurosci*, **2015**, 56(2), 320-328. doi: 10.1007/s12031-015-0536-3.
48. Sarkisova, K.Y.; Midzianovskaia, I.S.; Kulikov, M.A. Depressive-like behavioral alterations and c-fos expression in the dopaminergic brain regions inWAG/Rij rats with genetic absence epilepsy. *Behav. Brain Res*, **2003**, 144, 211–226. doi: 10.1016/s0166-4328(03)00090-1.
49. Naqvi, N.H.; Bechara, A. The hidden island of addiction: the insula. *Trends Neurosci*. **2009,** 32(1), 56-67. doi: 10.1016/j.tins.2008.09.009.
50. Krushinsky, L. V., Molodkina, L. N., Fless, D. A., Dobrokhotova, L. P., Steshenko, A. P., Semiokhina, A. F., ... & Romanova, L. G. (1970). The functional state of the brain during sonic stimulation. In *Physiological Effects of Noise: Based upon papers presented at an international symposium on the Extra-Auditory Physiological Effects of Audible Sound, held in Boston, Massachusetts, December 28–30, 1969, in conjunction with the annual meeting of the American Association for the Advancement of Science* (pp. 159-183). Springer US.
51. Fadyukova, O. E., Storozhevykh, T. P., Pinelis, V. G., Koshelev, V. B. (2004). Ischemic and hemorrhagic disturbances in cerebral circulation alter contractile responses of the rat middle cerebral artery. *Brain research*, *995*(1), 145-149. https://doi.org/10.1016/j.brainres.2003.09.062
52. Ruggiero, D. A., Mraovitch, S., Granata, A. R., Anwar, M., & Reis, D. J. (1987). A role of insular cortex in cardiovascular function. *Journal of Comparative Neurology*, *257*(2), 189-207. https://doi.org/10.1002/cne.902570206

53. Allen, G.V., Saper, C.B., Hurley, K.M., Cechetto, D.F., 1991. Organization of visceral and limbic connections in the insular cortex of the rat. J. Comp. Neurol. 311 (1), 1–16. https://doi.org/10.1002/cne.903110102.,
54. Gehrlach, D.A., Weiand, C., Gaitanos, T.N., Cho, E., Klein, A.S., Hennrich, A.A., Gogolla, N., 2020. A whole-brain connectivity map of mouse insular cortex. Elife 9. https://doi.org/10.7554/eLife.55585.
55. Livneh, Y., Andermann, M.L., 2021. Cellular activity in insular cortex across seconds to hours: Sensations and predictions of bodily states. Neuron 109 (22), 3576–3593. https://doi.org/10.1016/j.neuron.2021.08.036.
56. DuBois, D., Ameis, S. H., Lai, M. C., Casanova, M. F., & Desarkar, P. (2016). Interoception in autism spectrum disorder: A review. *International journal of developmental neuroscience*, *52*, 104-111
57. Muskens, J. B., Velders, F. P., & Staal, W. G. (2017). Medical comorbidities in children and adolescents with autism spectrum disorders and attention deficit hyperactivity disorders: a systematic review. *European child & adolescent psychiatry*, *26*(9), 1093-1103.