Current Neurobiology

Open Access Research Article

Metabolism of Serotonin in Genetically Determined Disorders of Dopamine Metabolism

Traktirov DS[1], Artemova VS[1], Nazarov IR[2], Karpenko MN[1], Pestereva NS[1*]

[1]*Department of Physiology, FSBSI Institute of Experimental Medicine, Saint-Petersburg, Russia*

[2]*Department of Medicine, Saint Petersburg State University, Saint-Petersburg, Russia*

ABSTRACT

Dopamine (DA) and Serotonin (5-HT) are the most significant neurotransmitters in the Central Nervous System (CNS). Separately, the physiological roles of DA and 5-HT have been studied in detail, and progress has been made in understanding their roles in normal and in various pathological conditions (Parkinson's disease, schizophrenia, addiction, depression, etc.) it has been shown that knockout of the gene encoding DAT leads not only to a significant redistribution of dopamine in the striatum, midbrain, prefrontal cortex, and hippocampus, but also to changes in the production of RNA enzymes of monoamine metabolism, as well as to serious changes in the level of serotonin, most clearly manifested in the cerebellum and spinal cord.

Keywords: DAT-KO; Dopamine; Serotonin; ADHD

HIGHLIGHTS

- The knockout of the gene encoding DAT leads not only to a significant redistribution of dopamine in various structures of the CNS, but also to changes in the production of RNA enzymes of monoamine metabolism.
- The knockout of the gene encoding DAT leads to serious changes in the level of serotonin, which is most pronounced in the cerebellum and spinal cord.
- Metabolism of serotonin in genetically determined disorders of dopamine metabolism.

INTRODUCTION

Dopamine (DA) and Serotonin (5-HT) are the most significant neurotransmitters in the Central Nervous System (CNS). Brain dopamine, predominantly synthezing in the Substantia Nigra pars compacta (SNpc), the Ventral Tegmental Area (VTA), and the arcuate nucleus of the hypothalamus, mainly regulates motor control, reward-based learning, arousal, addiction, activeness, motivation, and cognitive function. Serotoninergetic pathways beginning from dorsal and medial raphe nuclei innervate cortical and subcortical structures and determine the serotonin involvement in psychomotor inhibition, regulation of emotions and mood, cognition, and adaptation to stressors. Separately, the physiological roles of DA and 5-HT have been studied in detail, and progress has been made in understanding their roles in normal and in various pathological conditions (Parkinson's disease, schizophrenia, addiction, depression, etc.). However, there are very few papers researching interaction neurotransmitter systems, especially from a neurochemical point of view.

Received:	08-July-2023	**Manuscript No:**	JCNB-23-16925
Editor assigned:	10-July-2023	**PreQC No:**	JCNB-23-16925 (PQ)
Reviewed:	24-July-2023	**QC No:**	JCNB-23-16925
Revised:	03-January-2025	**Manuscript No:**	JCNB-23-16925 (R)
Published:	10-January-2025	**DOI:**	10.36648/JCNB.5.1.36

Corresponding author: NS Pestereva, Department of Medicine, Saint Petersburg State University, Saint-Petersburg, Russia; E-mail: pesterevans@yandex.ru

Citation: Traktirov DS, Artemova VS, Nazarov IR, Karpenko MN, Pestereva NS (2025) Metabolism of Serotonin in Genetically Determined Disorders of Dopamine Metabolism. J Curr Neur Biol. 5:36.

Meanwhile, functional disturbances in one of these systems necessarily will lead to “breakdowns” in the other system [1]. For example, a DA deficiency developing in Parkinson's Disease (PD) leads to the mental disorders development-anhedonia and loss of motivation. At the same time, the canonical PD treatment with L-dopa drugs contributes to the reduction of motor and non-motor symptoms of PD. Thus, it is obvious that DA is involved in the regulation of behavioral and mood conditions. There are examples of the opposite situation, when 5-HT plays the role of a powerful regulator of DA-dependent behavior. In addition to the undoubted functional connection between the DA and 5-HT systems, their anatomical and neurochemical intersections are shown. For example, 5-HT Transporters (SERT) can bind to DA and DA Transporter (DAT) binds to 5-HT. Therefore, animals with impaired DA metabolism may be a good model for studying the interaction of DA and 5-HT systems. Partially, this approach has already been tested in mice lacking the dopamine transporter (DAT-KO mice). DAT-KO mice are characterized by elevated extracellular DA and an abnormal D_1/D_2 ratio of dopamine receptors. These animals develop perseverative, compulsive, stereotypical, and hyperactive behaviors, which is indicative of 5-HT metabolism disorders [2]. Indeed, DAT-KO mice show an increase in 5-HT in the hippocampus and a decrease in the striatum. DAT-KO rats are characterized by a distinct set of behavioral disorders; they demonstrate pronounced stereotypy, deficit in working memory and fewer propensities to develop obsessive behaviors. Consequently, the changes in the 5-HT neurotransmitter system should be somewhat different in contrast to, but this issue has not been previously studied. Thus, the aim of this study was to investigate changes in the serotonin system, such as increased brain area-dependent changes in serotonin content and serotonin turnover rates, and changes mRNA content of serotonin metabolism enzyme in DAT-KO rats.

MATERIALS AND METHODS

Animals

The study was performed on DAT-KO (n=13) and DAT-WT (n=10) rats obtained from the vivarium of the institute of translational biomedicine, St. Petersburg State university [3]. All procedures with rats were carried out according to institutional guidelines and in compliance with the National Institutes of Health (NIH) guide for the care and use of laboratory animals and national laws (Russian Federation the Ministry of Health N267, June 19, 2003; guide for the use of laboratory animals, Moscow, 2005) were approved by the Local Ethics Committee of the Institute of Experimental Medicine, which follows the NIH guidelines for care and use of animals (ethical number №1/20 от 27.02.2020). The animals were housed in galvanized polypropylene cages of 5 rats each in a room with controlled conditions (24℃ ± 1℃ for temperature, 45-65% for humidity, and 12 h light/12 h dark cycle) [4]. In the period of the experiment, the pelleted rat chow and water were available ad libitum. After the start of the experiment, no animals were excluded.

Sample Collection

Rats were euthanized with Zoletil (15 mg/kg), after which they were decapitated with a guillotine (OpenScience AE1601, RPC OpenScience Ltd, Russia). Tissue samples were taken according to the brain atlas. Collected tissue was immediately frozen and stored at 80℃ until analysis [5].

Genotyping

The genotyping was performed after the experiments with rats. DNA was carrying out from fragments of tail tissue. Genotyping was performed by DAT gene DNA amplification with PCR followed by restriction enzyme BtsIMutI digestion and electrophoretic separation. The details of the method are described in Leo et al.

Determination of Catecholamines

For the quantification of monoamines (DA, DOPAC, HVA, 5-HT, 5-HIAA) brain tissue was homogenized in 0.1 M perchloric acid. The HPLC analysis was performed on a C18 reverse-phase column BDS Hypersil (250 × 4.6 mm, particle sz. 5 µm) under isocratic conditions with electrochemical detection. The mobile phase consisted of a 75 mM phosphate buffer containing 2 mM citrate acid, 0.1 mM octanesulfonic acid, and 5 % (v/v) acetonitrile (pH 3.1) [6]. Monoamine content was corrected for total protein concentration of the final sample, as assessed by the Pierce Bicinchoninic Acid (BCA) assay according to the manufacturer’s instructions (Thermo Fisher Scientific) and was expressed as ng/mg of total protein content using an external calibration curve.

RNA Isolation

Total RNA was isolated from brain regions using TRIzol reagent (Invitrogen, UK). RNA concentration was measured using a NanoDrop 2000 spectrophotometer (Thermo Scientific, USA) following the standard procedure. The purity of RNA samples was verified by confirming that each had an optical density ratio A260/A280>1.8. To verify the integrity of the samples, the 18S/28S RNA ratio was analyzed after electrophoresis in 1.4% agarose gel.

cDNA Synthesis and Real-Time RT-PCR

Two µg of total RNA was used for cDNA synthesis using high-capacityc DNA reverse transcription kits (applied biosystems). Quantitative real time RT-PCR was performed using Evrogen 5x qPCR mix–HS SYBR [7]. Primers were designed with the Primer-BLAST software (NCBI, USA); the primer sequences and sizes of PCR products are presented in the **Table 1**. GAPDH mRNA was used as an internal control. The delta-delta CT method was used to determine the fold increase of genes relative to the control group. The results are given as bar charts. Each value was combined from 3 independent PCR replicates for each cDNA samples, obtained from 5 animals.

Table 1: Primers used for RT-PCR.

S. no	Name	Primer sequence
1	MAO-A	Forward 5'-GCCAGGAACGGAAATTTGTA-3'; Reverse 5'-TCTCAGGTGGAAGCTCTGGT-3'
2	MAO-B	Forward 5'-TGGGCCAAGAGATTCCCAGTGATG-3'; Reverse 5'-AGAGTGTGGCAATCTGCTTTGTAG-3'
3	COMT130	Forward 5'-CTGGAGGCCATCGACACCTA-3'; Reverse 5'-AGTAAGCTCCCAGCTCCAGCA-3'
4	18S	Forward 5'-ACGGACCAGAGCGAAAGCAT-3'; Reverse 5'-TGTCAATCCTGTCCGTGTCC-3'
5	CYCR	Forward 5'-GGATTTGGCTATAAGGGTTC-3';Reverse 5'-GTTGTCCACAGTCGGAGA-3'

Statistical Analysis

Statistical analysis was conducted using Statistica 12.0 (StatSoft). Normality of the distribution was verified by the Shapiro-Wilk test. All data are expressed as the means ± SEM. Statistical differences were tested with the t-test. $p<0.05$ was considered statistically significant [8].

RESULTS

DA, 5-Ht and Their Metabolites in the Different Regions of CNS of DAT- KO Rats

The data are presented as mean ± SEM, t-test. DAT-KO (n=8) and DAT-WT (n=5)

We have analyzed the content of DA, 5-HT and its metabolites in the striatum, prefrontal cortex, hippocampus, medulla oblongata, cerebellum and spinal cord of DAT-KO rats (**Table 2**). Significant differences were detected for 5-HT and/or HIAA for all analyzed structures, meanwhile level of DA and/or it's metabolites distinction in control and DAT-KO rat's structure was observed everywhere in spite of celebellum. It turned out that in striatum, medulla oblongata and spinal cord of DAT- KO rats DA level was lower as compared to the control group at 8.5, 3.7, 5.0 times respectively (the corresponding p-values are given in the **Table 2**) [9].

There was DA higher at 2.3 times as compared to the control group in cortex and hippocampus of DAT-KO. In the striatum and cortex, we observed an increase in the content of DA metabolites: In the striatum DOPAC increased by 2.3 and HVA by 3.6 times; in the cortex the DOPAC content increased by 2.5 times with unchanged HVA compared with the control. In medulla oblongata, the content of DOPAC decreased by 3 times with unchanged HVA compared with the control [10]. The 5-HT level was decreased in cortex, medulla oblongata, cerebellum and spinal cord of DAT-KO rats by 2.4, 29.2, 13.5, 3.3 times, respectively, compared with the control group. We observed increase 5-HIAA content in the striatum and hippocampus by 2.6 and 1.1 times, respectively with the unchanging 5-HT level relative to the control group. At the same time, it was detected a decrease the 5-HT content was accompanied by a decrease in the content of its metabolite 5-HIAA in medulla oblongata and cerebellum by 3.4 and 2.0 times, respectively. In the cortex and spinal cord, against the background of a decrease in 5-HT, no signi icant changing the 5-HIAA content were found compared with the control group. In the midbrain, the DA level was reduced by 2 times in comparison with the control, the 5-HT level by 1.9 times.

Table 2: Concentrations of monoamines in the different brain regions of DAT-KO rats, ng/mg protein.

	DA	DOPAC	HVA	5-HT	5-HIAA
			Midbrain		
DAT-WT	5.1 ± 0.9	n.d.	n.d.	5.1 ± 0.0	0.1 ± 0.0
DAT-KO	2.8 ± 0.1	n.d.	n.d.	2.7 ± 0.1	0.3 ± 0.0
t	2.4			1.2	-5.4
p	0.012			0.008	0.003
			Striatum		
DAT-WT	64.3 ± 2.7	6.0 ± 0.7	3.8 ± 0.7	8.2 ± 0.8	4.2 ± 0.2
DAT-KO	7.5 ± 1.7	13.8 ± 1.7	13.8 ± 1.7	5.4 ± 1.3	11.1 ± 2.2
t	18.9	-3.7	-5.3	1.7	-2.6
p	0.0002	0.014	0.0075	0.1326	0.0428
			Cortex		

DAT-WT	0.8 ± 0.0	0.2 ± 0.0	0.3 ± 0.1	16.5 ± 2.7	13.1 ± 1.9
DAT-KO	1.8 ± 0.1	0.5 ± 0.1	0.3 ± 0.1	6.8 ± 0.7	9.7 ± 4.1
t	-16	-2.8	0.4	3.7	0.6
p	0	0.0271	0.673	0.0069	0.5264
Hippocampus					
DAT-WT	0.4 ± 0.0	0.2 ± 0.0	n.d.	13.1 ± 0.7	15.5 ± 0.2
DAT-KO	0.9 ± 0.1	0.2 ± 0.0	n.d.	13.6 ± 0.5	17.0 ± 0.2
t	-5	0.1		-0.6	-4.3
p	0.0043	0.9513		0.5432	0.0078
Medulla oblongata					
DAT-WT	1.1 ± 0.2	0.3 ± 0.1	0.4 ± 0.1	17.5 ± 3.6	13.4 ± 2.3
DAT-KO	0.3 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	3.9 ± 1.1
t	3.9	4.4	2.1	5.5	4
p	0.0057	0.0032	0.0742	0.0009	0.0052
Cerebellum					
DAT-WT	1.0 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	2.7 ± 0.9	1.6 ± 0.2
DAT-KO	0.7 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.8 ± 0.1
t	1.8	1.8	2.1	2.9	3
p	0.1229	0.1134	0.0742	0.022	0.0191
Spinal cord					
DAT-WT	0.5 ± 0.1	0.2 ± 0.0	0.3 ± 0.1	6.0 ± 0.6	4.4 ± 0.8
DAT-KO	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	1.8 ± 0.1	5.3 ± 0.9
t	3.4	1.4	1.1	7.9	-0.8
p	0.012	0.1889	0.3025	0.0001	0.4747

Turnover Rates of DA and 5-HT in the Different Brain Regions of DAT-KO Rats

The data are presented as mean ≠SEM, t-test.

We calculated the ratio of DOPAC to DA to index intracellular DA turnover; we calculated the ratio of HVA to DA to DA to index extracellular DA turnover [11]. It was shown, intracellular DA turnover rates in the striatum and spinal cord of DAT-KO rats is lower by 20 and 2 times respectively in comparison to control group the corresponding p-values are given in the **Table 3**, meanwhile extracellular DA metabolism was intensified only in the striatum (by 38 times). On the contrary, the intracellular DA metabolism rate was decreased in the hippocampus and medulla oblongata of DAT-KO rats by 2.0 and 1.5 times, respectively, relative to the control. The extracellular DA rate metabolism in the hippocampus could not be determined, and in the medulla oblongata of DAT-KO rats it was comparable to the control. While in the cortex intracellular DA metabolism rate was unchanged relative to the control, however the rate of extracellular metabolism was 4 times lower [12].

We calculated the ratio of 5-HIAA to 5-HT to index 5-HT turnover. It turned out that the turnover rates of 5-HT in the striatum, cerebellum and spinal cord of DAT-KO rats increased relative to the control by 4.2, 5.0 and 4.4 times, respectively. DAT-KO turnover rates of 5-HT in the cortex and hippocampus were comparable to the control group counts. The most medulla oblongata samples contain too small 5-HT to analyze it.

Table 3: Turnover rates of DA and 5-HT in the different brain areas of DAT-KO rats, A.U.

	DOPAC/DA	HVA/DA	5-HIAA/5-HT
Striatum			
DAT-WT	0.1 ± 0.0	0.1 ± 0.0	0.5 ± 0.0
DAT-KO	2.0 ± 0.2	3.8 ± 0.4	2.1 ± 0.1
t	-9.7	-9.2	-10.7
p	0.0023	0.0027	0.0012
Cortex			
DAT-WT	0.3 ± 0.1	0.4 ± 0.1	0.9 ± 0.2
DAT-KO	0.3 ± 0.0	0.1 ± 0.1	1.5 ± 0.6
t	0.2	2.4	-0.9
p	0.82	0.0475	0.4201
Hippocampus			
DAT-WT	0.6 ± 0.1	n.d.	1.2 ± 0.0
DAT-KO	0.3 ± 0.0	n.d.	1.3 ± 0.0
t	4.3		-1.2
p	0.0075		0.2918
Medulla oblongata			
DAT-WT	0.3 ± 0.0	0.4 ± 0.1	0.9 ± 0.3
DAT-KO	0.2 ± 0.0	0.5 ± 0.1	n.d.
t	3.5	-1.3	
p	0.0094	0.2309	
Cerebellum			
DAT-WT	0.1 ± 0.0	0.1 ± 0.0	0.7 ± 0.2
DAT-KO	0.2 ± 0.1	0.2 ± 0.1	3.5 ± 0.3
t	-0.6	-0.9	-6.9
p	0.5745	0.3592	0.0002
Spinal cord			
DAT-WT	0.4±0.2	0.7±0.4	0.7±0.1
DAT-KO	0.8±0.1	0.6±0.4	3.1±0.7
t	-1.7	0.1	-2.8
p	0.1272	0.8975	0.0281

mRNA Expression Level of the Main Monoamine Metabolism Enzymes in Different CNS Regions of DAT-KO rats

We determined mRNA levels of the MAO-A, MAO-B and COMT genes in striatum, cortex, hippocampus, medulla oblongata, cerebellum and spinal cord of DAT-KO rats (**Table 4**). It was discovered the lower level of MAO-A, MAO-B mRNA expression by 2.5 and 5 imes respec ively in the striatum of DAT-KO rats compared to control animals the corresponding p-values are given in the **Table 4** [13]. The same differences were detected for medulla oblongata-content of MAO-A

mRNA has been lowered by 12.0 times, MAO-B-10.0 times, COMT-5.0 times for DAT-KO rats. Conversely, the mRNA level has been higher: In the cortex by 2.1 times for MAO-A, by 2.0 times for MAO-B; in the cerebellum mRNA level of COMT was increased by 5.0 times-for DAT-KO rats versus control rats. There was a decreased mRNA level of MAO-A by 1.2 times, increased mRNA levels of MAO-B by 13 times and of COMT bz 10.8 times in the sample of DAT-KO spinal cord compared control. In the hippocampus significant differences were shown for MAO-B mRNA content it was higher for DAT-KO samples by 2.2 times in comparison to control rat samples.

Table 4: Normalized relative gene expression of MAO-A, MAO-B and COMT mRNA in the different CNS regions of DAT-KO rats, A.U.

	MAO-A	MAO-B	COMT
Striatum			
DAT-WT	1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.1
DAT-KO	0.4 ± 0.1	0.2 ± 0.1	1.0 ± 0.2
t	7.4	6.4	0.2
p	0.0003	0.0002	0.8688
Cortex			
DAT-WT	1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.1
DAT-KO	2.1 ± 0.2	2.0 ± 0.4	1.2 ± 0.2
t	-4.2	-2.3	-0.8
p	0.0031	0.0454	0.4283
Hippocampus			
DAT-WT	1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.1
DAT-KO	0.6 ± 0.2	2.2 ± 0.5	2.1 ± 0.7
t	1.8	-2.7	-1.6
p	0.1104	0.029	0.1561
Medulla oblongata			
DAT-WT	1.2 ± 0.2	1.0 ± 0.2	1.0 ± 0.1
DAT-KO	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.1
t	5.8	3.9	4.5
p	0.0004	0.0048	0.0015
Cerebellum			
DAT-WT	0.1 ± 0.0	0.1 ± 0.0	0.7 ± 0.2
DAT-KO	0.2 ± 0.1	0.2 ± 0.1	3.5 ± 0.3
t	-0.6	-0.9	-6.9
p	0.5745	0.3592	0.0002
Spinal cord			
DAT-WT	1.1 ± 0.1	1.0 ± 0.1	1.0 ± 0.1
DAT-KO	0.9 ± 0.1	13.0 ± 2.0	10.8 ± 3.5
t	0.8	-5.8	-2.8

p	0.4301	0.0004	0.024

The data are presented as mean ± SEM, t-test. DAT-KO (n=5) and DAT-WT (n=5)

DISCUSSION

The most studied and functionally important dopaminergic tract is the nigrostiate tract. It begins in the compact zone of the substantia nigra and extends into the striatum, the axons of its neurons release about 80% of dopamine in the CNS. In the midbrain of DAT-KO rats: The level of dopamine was reduced by 2 times compared with the control; the level of serotonin was lower by 1.9 times (**Table 2**). The trend also spread to the striatum, where the DA content decreased by 8.6 times in the KO group. The results obtained for the striatum are consistent with the results of our colleagues: The level of DA of DAT-KO rats is much lower than in the control group (by 13 times), and the level of dopamine metabolites-DOPAC and HVA-is higher than in the control (by 1.2 and 2.5 times, respectively). According to the same work, the level of extracellular dopamine in the striatum of DAT-KO rats is 8 times higher than in WT rats [14]. An explanation can be found in the work of Mohamed Jaber, who showed a decreasing expression mRNA of Tyrosine Hydroxylase (TH) by 25% in the ventral midbrain of DAT-KO mice. The level of TH protein expression in this structure was reduced by 35%, however, in the striatum, the level of TH protein expression was reduced by 90% in comparison with the control. We suggest that due to a genetically determined increase in the extracellular level of dopamine, its synthesis can be reduced saving cell resources and therefore the expression of tyrosine hydroxylase, the enzyme that metabolizes tyrosine into L-dopa (DA precursor), has decreased. We also showed a simultaneous increase in the level of metabolites with a decrease in DA: DOPAC by 2.3 times; HVA by 3.6 times [15]. Thus, the conversion of metabolites increased by 20 (DOPAC/DA) and 38 (HVA/DA) times, respectively. In addition, the conversion of 5-HT to 5-HIAA increased 4-fold. The main reason for the increase in DA and 5-HT conversion is a change in the functioning of the enzyme system that metabolizes monoamines. The key representatives are MAO-A, MAO-B, COMT.

We have analyzed changes in the level of expression of mRNA of these enzymes. It was shown by PCR that in the midbrain the expression levels of mRNA MAO-A (by 4.8 times), MAO-B (by 1.3 times), and COMT (by 4.2 times) were significantly reduced. It was shown that in the striatum the expression levels of mRNA MAO-A (by 2.5 times) and MAO-B (by 5 times) significantly decreased (**Table 3**) [16]. These results are logical from the terms of adaptation processes: Due to the knockout of the gene encoding DAT, the level of total DA decreases, which means that the number of enzymes that metabolize it can also be reduced. However, it remains unclear due to what mechanisms the conversion increases? It can be assumed that the mechanisms of post-translational modifications of MAO-A,-B proteins are involved, leading to an increase in the stability of the corresponding proteins or an increase in their enzymatic activity.

The second dopaminergic pathway of interest is the mesocortical one. It connects the ventral tegmentum of the midbrain with the frontal lobe of the cerebral cortex, predominantly with the prefrontal cortex. This pathway is important for normal cognitive functioning, and the mesocortical pathway is involved in regulation of motivational and emotional response processes. For the cortex, the following changes were shown: The levels of DA and DOPAC were increased (by 2.5 and 2.3 times, respectively) in comparison with the control [17]. Therefore, the HVA/DA conversion decreased by 4 times. The level of 5-HT in the cortex was 2.4 times higher (**Table 2**). mRNA expression levels were increased in comparison with WT MAO-A (2.1 times) and MAO-B (2 times) (Table 3). This confirms the plasticity and high adaptive ability of the dopaminergic system: Due to the knockout, the level of extracellular dopamine increased-the production of mRNA enzymes for its cleavage increased. Based on the data, that DA has a higher affinity for Norepinephrine Transporters (NETs) than for DAT and the density of Prefrontal cortex (PFc) DAT is relatively low compared to the density of PFc NET, this data explains the unchanged level of HVA: The main conversion of HVA/DA occurs outside the cell, and since DA was taken aback by NET, no changes were observed in this pathway.

Next, the dopamine innervation of the hippocampus was considered. With disorders associated with dysregulation of the functioning of the dopaminergic system, there are deterioration's in both working and spatial memory, the formation of which is responsible for the hippocampus. The level of DA in DAT-KO rats was increased by 2.3 times, due to which the conversion of DOPAC/DA decreased by 2 times in the hippocampus. It was shown that the levels of 5-HT and 5-HIAA remained almost unchanged. The expression level of MAO-B in knockout animals was 2.2 times higher, which is explained by adaptation to an increased content of dopamine due to reduced reuptake [18,19]. In addition, an increase in the NE level in the hippocampus of mice with DAT gene knockout was shown, which was associated with antisocial behavior, which is observed in DAT-KO animals. MAO-B, among other things, regulates metabolism in the adrenergic system; accordingly, along with an increase in the level of NE, MAO-B expression also increased.

The most significant changes in the content of serotonin and its metabolites were shown for the cerebellum and spinal cord. In the cerebellum, the level of serotonin decreased by 13.5 times, the level of 5-HIAA decreased by 2 times, respectively, the conversion of 5-HT to 5-HIAA increased by 5 times. Recent work has shown that the cerebellum regulates a number of different functions in addition to motor functions, such as working memory, emotion, response timing, action planning, and attentional control. Interestingly, impairment of

these functions has been found in Autism Spectrum Disorders (ASD) and Attention Deficit Hyperactivity Disorder (ADHD). Both disorders are closely related to each other, overlap in genetic vulnerability, and share similar patterns of social impairment and increased antisocial behavior. DAT-KO rats are a recognized model of Attention Deficit Hyperactivity Disorder (ADHD), so the neurochemical changes in the cerebellum that we have identified should be taken into account in further studies of ADHD and ASD. It is consistent with our results that the ability to synthesize 5-HT decreases with age in children with ASD.

In the spinal cord, the DA level in knockout animals was 5-fold lower with unchanged DOPAC levels, due to which DOPAC/DA conversion increased 2-fold; the level of 5-HT decreased by 3.33 times with the unchanged level of 5-HIAA, the conversion of 5-HIAA/5-HT increased by 4.4 times. Expression of mRNA of DAT-KO enzymes in rats in the spinal cord was increased: MAO-B- in 13 times, COMT-10.8 times. Monoamine oxidases are involved in the metabolism of not only dopamine and serotonin, but also the monoamines of the adrenergic system. We assume that in this case, the transmission of adrenaline/noradrenaline increases, thus, there is a need for their more active utilization.

Neurotransmitter systems are in close morphological and functional contact with each other. In this work, we have shown the knockout of the gene encoding DAT leads not only to a significant redistribution of dopamine in various structures of the CNS, but also to changes in the production of RNA enzymes of monoamine metabolism, as well as to serious changes in the level of serotonin, which is most pronounced in the cerebellum and spinal cord. The crucial changes we identified are summarized in **Figure 1**. These data should be taken into account when using dopamine reuptake inhibitors (Bupropion) in clinical practice, as well as when prescribing monoamine oxidase blockers.

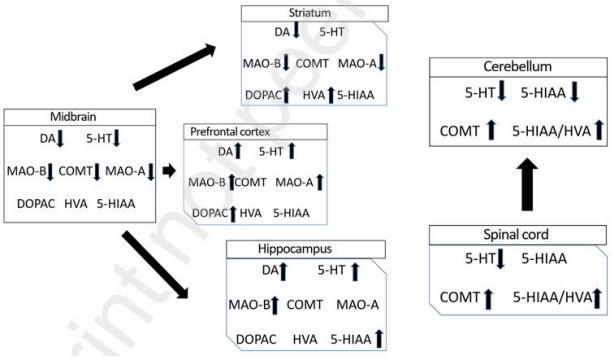

Figure 1: Changes caused by knockout of the *DAT* gene.

CONCLUSION

We see that changes in the midbrain, cortex, and hippocampus can be fully explained by adaptive mechanisms that are included in the work of the dopaminergic system due to knockout of the dopamine reuptake transporter gene. The change in the monoamine composition in the striatum is not subject to this theory, however, various studies of the behavior of DAT-KO animals show their differences from the control, for example, problems with concentration (Morris water maze), constant movement (Open field), which may well be due to dysregulation work of the dopaminergic system in the striatum. The accumulation of a large amount of dopamine in the synaptic cleft can lead to its oxidation and the formation of a toxic form-oxidopamine, which will be oxidative stress for the cell and will negatively affect the homeostasis of this and neighboring cells. Similar conclusions are also valid for the cerebellum, which is confirmed by literature data, including works on the study of ASD and ADHD.

LIMITATIONS

The brilliant addition to our research to analyze enzyme activity in the CNS samples. However routine classic method what used for this aim is more suitable for blood samples than for brain ones. The necessary sensibility can be reached by commercial kit, but in this experiment, the sample amount was not enough to analyze the activity. The second addition what can upgrade this research is measuring the level of NET RNA in the cortex. We want to do it in the continue this research.

REFERENCES

1. Adinolfi A, Zelli S, Leo D, Carbone C, Mus L, et al. Behavioral characterization of DAT-KO rats and evidence of asocial-like phenotypes in DAT-HET rats: The potential involvement of norepinephrine system. Behav Brain Res 359:516-527.
2. Bacque Cazenave J, Bharatiya R, Barriere G, Delbecque JP, Bouguiyoud N, et al. (2020) Serotonin in animal cognition and behavior. Int J Mol Sci 21(5):1649.
3. Baronti F, Davis TL, Boldry RC, Mouradian MM, Chase TN (1992) Deprenyl effects on levodopa pharmacodynamics, mood, and free radical scavenging. Neurology 42:541-544.
4. Bruchhage MM, Bucci MP, Becker EB (2018) Cerebellar involvement in autism and ADHD. Handb Clin Neurol 155:61-72.
5. DC C (2005) Developmental changes in brain serotonin synthesis capacity in autistic and nonautistic children. Am J Hum Genet. 77(2):265-279.
6. Daw ND, Kakade S, Dayan P (2002) Opponent interactions between serotonin and dopamine. Neural Netw 15:603-616.
7. Devroye C, Cathala A, Piazza PV, Spampinato U (2018) The central serotonin2B receptor as a new pharmacological target for the treatment of dopamine-related neuropsychiatric disorders: Rationale and current status of research. Pharmacol Ther 181:143-155.
8. Eshleman AJ, Carmolli M, Cumbay M, Martens CR, Neve KA, et al. (1999) Characteristics of drug interactions with recombinant biogenic amine transporters expressed in the same cell type. J Pharmacol Exp Ther 289(2):877-885.

9. Fox MA, Panessiti MG, Hall FS, Uhl GR, Murphy DL (2013) An evaluation of the serotonin system and perseverative, compulsive, stereotypical, and hyperactive behaviors in Dopamine Transporter (DAT) knockout mice. Psychopharmacology 227:685-695.
10. Franco R, Reyes-Resina I, Navarro G (2021) Dopamine in health and disease: much more than a neurotransmitter. Biomedicines 9(2):109.
11. van Galen KA, Ter Horst KW, Booij J, la Fleur SE, Serlie MJ (2018) The role of central dopamine and serotonin in human obesity: lessons learned from molecular neuroimaging studies. Metabolism 85:325-339.
12. Hao H, Shao M, An J, Chen C, Feng X, et al. (2014) Association of Catechol-O-Methyltransferase and monoamine oxidase B gene polymorphisms with motor complications in parkinson's disease in a Chinese population. Parkinsonism Relat Disord 20(10):1041-1045.
13. Ito M (2013) Error detection and representation in the olivo-cerebellar system. Front Neural Circuits. 7:1.
14. Jaber M, Dumartin B, Sagne C, Haycock JW, Roubert C, et al. (1999) Differential regulation of tyrosine hydroxylase in the basal ganglia of mice lacking the dopamine transporter. Eur J Neurosci 11(10):3499-3511.
15. Kapur S, Remington G (1996) Serotonin-dopamine interaction and its relevance to schizophrenia. Am J Psychiatry 153(4):466-476.
16. Kurzina NP, Aristova IY, Volnova AB, Gainetdinov RR (2020) Deficit in working memory and abnormal behavioral tactics in dopamine transporter knockout rats during training in the 8-arm maze. Behav Brain Res 390:112642.
17. Larsen MB, Sonders MS, Mortensen OV, Larson GA, Zahniser NR, et al. (2011) Dopamine transport by the serotonin transporter: A mechanistically distinct mode of substrate translocation. J Neurosci 31(17):6605-6615.
18. Leo D, Sukhanov I, Zoratto F, Illiano P, Caffino L, et al. (2018) Pronounced hyperactivity, cognitive dysfunctions, and BDNF dysregulation in dopamine transporter knock-out rats. J Neurosci 38(8):1959-1972.
19. Lerner TN, Holloway AL, Seiler JL (2021) Dopamine, updated: Reward prediction error and beyond. Curr Opin Neurobiol 67:123-130.