Cell. Mol. Biol. (ISSN: 1165-158X)

Journal Homepage: www.cellmolbiol.org

Cellular and Molecular Biology

Original Article

Regulation of calpain 1 and calpain 2 activity by dopamine in rat brain regions

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Article Info

Article history:

Received: November 07, 2025
Accepted: February 06, 2026
Published: February 28, 2026

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Abstract

Calpains are involved in regulating various brain functions. Limited proteolysis regulates cell proliferation, apoptosis, synaptic plasticity, long-term potentiation, and cell signaling. These are only some of the processes regulated by calpains. The current study reveals previously unexplored states of calpain activation associated with disorders of the dopaminergic system, including both hyperfunctional and hypofunctional states. In addition, activation of calpain 1 and calpain 2 affects the functioning of the central nervous system differently. Thus, this work aimed to study the effect of dopamine deficiency or excess on the activity of calpain 1 and calpain 2 in various parts of the central nervous system. We used L-dopa administration and DAT-KO rats to model a hyperdopaminergic state, and AMPT and reserpine to model a hypodopaminergic state. Dopamine deficiency causes: increased activity of calpain 1 in the striatum after AMPT administration; decreased activity of calpain 2 in the hippocampus after L-dopa administration. Excess dopamine causes: increased activity of calpains 1 and 2 in the striatum after L-dopa administration; decreased activity of calpains 1 and 2 in the midbrain after reserpine administration. Our data show that DAT knockout is associated with increased dopamine levels and increased activity of calpain 2 in the hippocampus, as well as decreased dopamine levels in the striatum and in the midbrain, accompanied by increased calpain 1 activity in the striatum.

Keywords: Dopamine, Calpains, Reserpine, AMPT, L-dopa, DAT-KO, Rats.

1. Introduction

Dopamine, a member of the catecholamine group along with norepinephrine and epinephrine, is a key neurotransmitter involved in the regulation of physiological processes such as motor control, motivation, reward, cognitive function, as well as maternal and reproductive behavior [1-5]. Dysfunction of the dopaminergic system through various molecular pathways leads to the development of movement disorders, compulsive behavior, the formation of addictions, disruption of neuroendocrine regulation mechanisms, dysregulated metabolic disorders, etc. [6-8]. Therefore, to understand the pathogenesis of dopamine-dependent conditions, it is necessary to study in detail the molecular pathways underlying the development of a particular dysfunction.

To date, it is believed that the main effects of dopamine are realized through its interaction with D1-like family and D2-like family receptors with subsequent activation of various systems of intracellular mediators [9]. However, disruption of the vesicular transporter VMAT2, for example, due to disruption of the proton gradient, can lead to the release of dopamine into the cytosol, where at neutral pH it undergoes protonation at the amino group to form the dopamine quinone [10]. The quinone is highly reactive and interacts with cellular components, for example, forming covalent modifications with proteins at cysteine residues [11]. Therefore, the functions of dopamine can be realized not only through its interaction with receptors. For example, dopamine can be involved in the regulation of the activity of calpains, calcium-dependent neutral cysteine proteases of the calpain system [12]. The calpain system is represented by calpains (from the words "calmodulin" and "papain") and calpastatin (an endogenous, highly specific inhibitor of calpains). To date, fifteen proteases of this family are known (calpain 1 - calpain 15), which have different tissue specificity and are activated at different levels of calcium in the cell. The most studied are calpain 1 and calpain 2, or, in another classification, m- and mu-calpain. Both forms of calpain have similar biochemical characteristics, but their activation occurs at different concentrations of Ca^{2+} ions: mu-calpain requires a micromolar concentration of Ca^{2+}, m-calpain – a millimolar concentration [12]. Although dopamine affects calpains *in vitro*, the *in vivo* consequences of dopamine deficiency/excess on calpain 1 & calpain 2 in different brain regions are unknown. More than 100 calpain substrates have been identified, and calpain-mediated cleavage alters their functions. Calpains participate in the regulation of synap-

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Doi: http://dx.doi.org/10.14715/cmb/2025.72.2.3

tic transmission, long-term potentiation, signal transduction, etc. [13]. Recent data have shown that the ubiquitous calpain 1 and calpain 2 perform opposite functions in the brain, probably due to different activation conditions and specificity of distribution in the cell, even though they do not have high substrate specificity. It has been proven that activation of calpain 1 is necessary for induction of synaptic plasticity and promotion of development of plastic processes, and that calpain 2 disrupts plasticity processes and is an inducer of neurodegeneration [14]. Consequently, cytosolic dopamine or dopamine quinone, modifying the activity of calpain 1 or calpain 2, can also interfere with the development of pathological processes through this pathway. This assumption is based on the first approximation of the study by K. Yurko-Mauro & E. Friedman, who showed that incubation of surviving hippocampal slices in the presence of 1 nM dopamine leads to an increase in the content of autolyzed calpain 2. It indicates the activation of calpain 2 during such incubation [15]. Treatment of human endothelial cells HUVEC with 25 μM dopamine resulted in suppression of hyperactivation of calpain 1, but not calpain 2, which was induced by cold stress [16]. Thus, depending on the object, incubation conditions and concentration of dopamine in the medium, multidirectional effects of dopamine on the activity of various representatives of calpains were observed. This work aimed to study the effect of dopamine deficiency or excess on the activity of calpain 1 and calpain 2 in various parts of the central nervous system.

2. Materials and Methods

2.1 Animals

Male Wistar rats (200±20 g) were purchased from the Rappolovo nursery (Leningrad Region, Russia). Male DAT-KO rats (200±20 g) were purchased from the Rappolovo nursery (Leningrad Region, Russia). The animals were housed in cages in a room with controlled conditions, including a temperature of 24±1 °C, 45–65% humidity, and 12 h light/12 h dark cycle. In the experimental period, pelleted rat chow and water were available *ad libitum*. 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) and certified by the local ethics committee of the Institute of Experimental Medicine (Ethics Issue No. 1/20, 2020). The rats were decapitated using a guillotine (OpenScience AE1601, OOO SPC OpenScience, Russia).

2.2 Reagents

Methyl L-dopa hydrochloride (D1507, Sigma-Aldrich), α-Methyl-DL-tyrosine methyl ester hydrochloride (M3281, Sigma-Aldrich), Reserpine (83580, Sigma-Aldrich).

2.3 Dopamine imbalance modeling

The experimental design is presented in Fig. 1.

2.4 Calpain activity detection

Calpain activity was analyzed according to the method described in [17]. In each experiment, the hippocampus, striatum, and midbrain were homogenized in Tissue Lysis Buffer (pH 7.4; Promega) and centrifuged at 12,000 × g for 30 minutes at 4°C. Total protein concentration was quantified via the Bio-Rad DC protein assay, calibrated against bovine serum albumin standards. A discontinuous gel system was employed, comprising a 4% acrylamide stacking gel and a 9% acrylamide separating gel copolymerized with 0.1% casein. Following a 45-minute equilibration phase, aliquots containing 50 μg of protein were electrophoresed at 125 V for 2.5 hours at 4°C in standard running buffer. Post-electrophoresis, gels were stained with Coomassie Brilliant Blue (Hi Media). Unstained regions corresponding to μ-calpain (upper zone) and m-calpain (lower faint zone) were identified, with enzymatic activity quantified based on the proportional area of unstained bands. Gel images were acquired using a Gel Doc XR+ system (Bio-Rad) under backlight illumination, and band areas were analyzed via the MultiSpec software (https://mygeohub.org/resources/multispec) following established protocols [17, 23]. A representative zymogram is shown in Fig. 2.

2.5 Dopamine detection

Quantitative analysis of monoamines was performed using the HPLC-ED method. Sample preparation: a portion of the brain structure of interest was homogenized in 40% $HClO_4$, then centrifuged at 12,000 g for 30 minutes. The supernatant in a volume of 20 μl was introduced into the chromatographic system. The HPLC system is equipped with an analytical column BDS HYPERSIL C18 (28105-259070A, manufacturer Thermo Scientific), a DECADE II SCC detector with an analytical cell and a protective casing (manufacturer ANTEC LEYDEN). The following were used as the mobile phase: 10 mM phosphate buffer pH = 3.10, 5% acetonitrile, 5% tetrahydrofuran.

Calibration was performed using the external standard method. Solutions containing dopamine in pre-prepared concentrations (10 ng/ml, 20 ng/ml, 30 ng/ml) were used as a standard. The amount of dopamine was normalized to the amount of total protein in the sample.

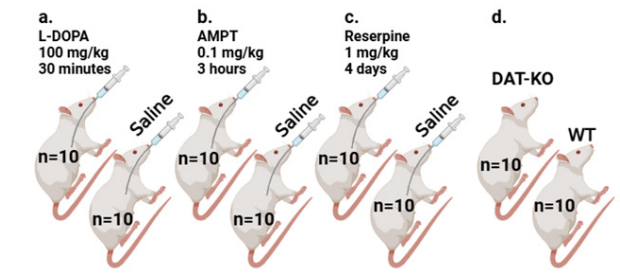


Fig. 1. Scheme of dopamine imbalance modeling.

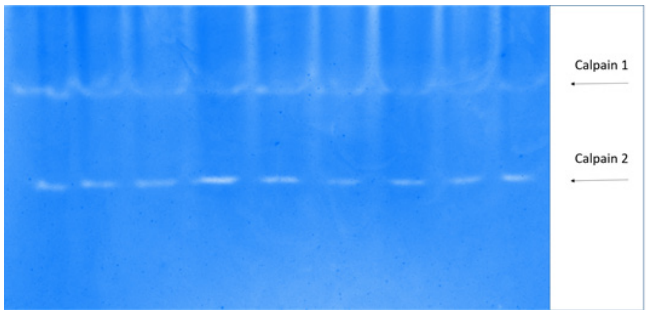


Fig. 2. Representative zymogram.

2.6 Measuring protein concentration in a sample using a drop spectrophotometer

The sediment obtained at the stage of sample preparation for HPLC was resuspended in 1M NaOH, centrifuged for 20 minutes at 12,000 g, after which the protein concentration in the supernatant was measured using a NanoDrop 2000 drop spectrophotometer. Samples were applied in a volume of 2 µl. Measurements were performed at a wavelength of 280 nm.

2.7 Statistical analysis

Statistical analysis was conducted using the GraphPad Prism 8.0 software (GraphPad Software, USA). The normality of distribution was tested by the Shapiro-Wilk test. All data are expressed as mean ± SD. Statistical hypotheses were tested using Student's t-test for independent samples. Significance level was set at $p < 0.05$. The overall weighted effect size (Cohen's d) for all studies was 0.4 or higher, a significant medium to large effect.

3. Results

3.1 Effects of L-dopa administration

After administration of L-dopa (100 mg/kg), the HPLC analysis revealed an increase in dopamine in the striatum by 1.6 ± 0.4 times ($p < 0.05$), which was a control indicator of the successful administration of L-dopa and the dopamine synthesis directly in the cells of the central nervous system of the animal. Dopamine in the hippocampus decreased by 1.9 ± 0.2 times ($p < 0.05$) (Fig.3).

The method of casein zymography in gel showed that the administration of L-dopa to animals led to an increase in the calpain 1 activity by 1.3 ± 0.1 times ($p < 0.05$) and calpain 2 by 1.8 ± 0.2 times ($p < 0.01$) in the cells of the striatum (Table 1) relative to the control group. A decrease in calpain 2 activity by 1.8 ± 0.1 times ($p < 0.01$) was shown in hippocampal cells in comparison to control animals (Table 1). In the midbrain cells of animals that received L-dopa, an increase in the activity of both forms of calpains was observed: calpain 1 by 1.5 ± 0.2 times ($p < 0.02$), calpain 2 by 1.6 ± 0.1 times ($p < 0.01$) (Fig.3).

3.2 Effects of AMPT administration

The second stage consisted of the study of the effect of dopamine deficiency on calpain activity. Intracellular dopamine deficiency was induced using AMPT (Alpha-methyl-p-tyrosine). AMPT is an inhibitor of tyrosine hydroxylase, an enzyme that metabolizes tyrosine into L-dopa, which results in a decrease in dopamine levels. After AMPT administration, dopamine content significantly decreased only in the striatum by 2.7 ± 0.1 times ($p < 0.05$)

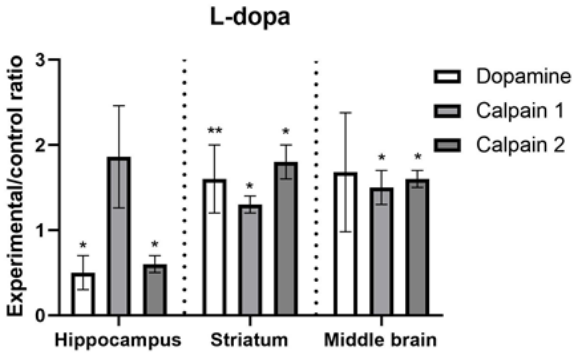


Fig. 3. Dopamine content and calpain activity in CNS cells after a single administration of L-dopa at a dose of 100 mg/kg. * - $p<0.05$, ** - $p\leq0/01$. The results are presented as the ratio of the experiment to the control. Absolute values and statistical data are presented in Table 1 of the supplementary materials.

Table 1. Dopamine content and calpain activity in CNS cells after a single administration of L-dopa at a dose of 100 mg/kg. The results are presented as the ratio of the experiment to the control.

		Dopamine	Calpain 1	Calpain 2
Hippocampus	Control	0.64 ± 0.07	62.67 ± 12.57	214 ± 13.09
	Experiment	0.33 ± 0.072	116.33 ± 14.11	123 ± 6.93
	Experiment/Control	0.5 ± 0.2 (p=0.019, t=2.447)	1.86 ± 0.6 (p=0.09, t=2.776)	0.6 ± 0.1 (p=0.018, t=3.182)
Striatum	Control	59.03 ± 10.13	1775 ± 107.002	1418.67 ± 63.25
	Experiment	97.13 ± 21.46	2384 ± 25	2547 ± 160
	Experiment/Control	1.6 ± 0.4 (p=0.01, t=2.571)	1.3 ± 0.1 (p=0.04, t=3.182)	1.8 ± 0.2 (p=0.017, t=3.182)
Midbrain	Control	66.95 ± 11.33	955.67 ± 29.77	2167.33 ± 69.03
	Experiment	112.15 ± 26.03	1435 ± 117.39	3425.33 ± 161.79
	Experiment/Control	1.68 ± 0.7 (p=0.25, t=2.447)	1.5 ± 0.2 (p=0.038, t=4.303)	1.6 ± 0.1 (p=0.016, t=3.182)

Table 2. Dopamine content and calpain activity in CNS cells after a single administration of AMPT at a dose of 100 µg/kg. The results are presented as the ratio of the experiment to the control.

		Dopamine	Calpain 1	Calpain 2
Hippocampus	Control	0.36 ± 0.57	179.18 ± 11.29	242.38 ± 6.11
	Experiment	0	184.78 ± 5.95	246.46 ± 3.32
	Experiment/Control	N/A	1.0 ± 0.1 (p=0.519, t=2.447)	1.0 ± 0.6 (p=0.227, t=2.365)
Striatum	Control	35.28 ± 4.65	155.73 ± 10.88	236.27 ± 9.82
	Experiment	13.063 ± 2.717	170.55 ± 7.45	235.69 ± 3.87
	Experiment/Control	0.37 ± 0.1 (p=0.00004, t=2.447)	1.1 ± 0.0 (p=0.05, t=2.306)	1.0 ± 0.4 (p=0.512, t=2.776)
Midbrain	Control	3.13±0.67	47.05±1.195	60.02±2.91
	Experiment	6.41±4.32	45.41±2.364	54.9±4.38
	Experiment/Control	2.05 ± 1.8 (p=0.35, t=0.8751)	1.0 ± 0.2 (p=0.171, t=2.447)	0.91 ± 0.1 (p=0.041, t=2.571)

(Table 2).

The results of casein zymography (Fig.4) are as follows: calpain 1 activity in the striatum increased by 1.1 ± 0.0 times. In the midbrain, calpain 2 activity decreased by 1.1 ± 0.1 times ($p < 0.05$).

3.3 Effects of reserpine administration

The study of extracellular dopamine deficiency was continued in the following experiment: reserpine was administered orally to the experimental group (1 mg/kg daily, 4 days). Reserpine irreversibly blocks H^+-linked vesicular monoamine transporters, VMAT1 and VMAT2. A significant change in the dopamine levels in the experimental animals was detected only in the midbrain. It increased by 1.6 ± 0.8 times ($p < 0.05$) (Table 3).

After a course of reserpine, calpain activity in the midbrain decreased: calpain 1 by 1.3 ± 0.3 times, calpain 2 by 1.5 ± 0.3 times (Fig. 5).

3.4 Effects of DAT knockout

The final stage of the *in vivo* experiments was conducted on DAT-KO rats, which have a genetic mutation that causes a significant accumulation of dopamine in the synaptic cleft. The control group consisted of 10 Wistar rats, while the experimental group included 10 DAT-KO rats. There were significant differences in dopamine levels in the hippocampus and striatum between the two groups. In the experimental rats, the dopamine level was 2.4 ± 0.4 ($p < 0.01$) times higher in the hippocampus and 3.5 ± 0.11 ($p < 0.048$) times lower in the striatum, compared to the control group (Table 4).

The activity of calpain 1 was increased by 1.82 ± 0.33 ($p=0.05$) in the striatum of the DAT-KO group ($p < 0.05$).

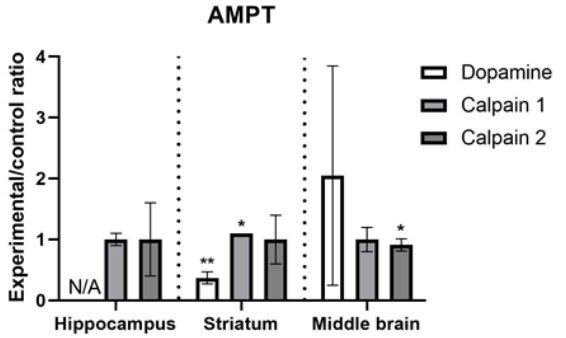


Fig. 4. Dopamine content and calpain activity in CNS cells after a single administration of AMPT at a dose of 100 μg/kg. * - $p<0.05$, ** - $p\leq0/01$. The results are presented as the ratio of the experiment to the control. Absolute values and statistical data are presented in Table 2 of the supplementary materials.

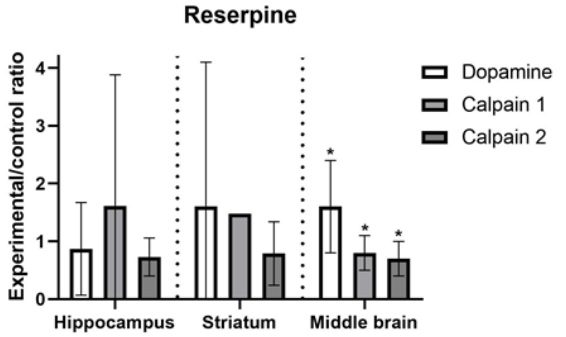


Fig. 5. Dopamine content and calpain activity in CNS cells after a course of reserpine administration at a dose of 1 mg/kg daily for 4 days. * - $p<0.05$, ** - $p\leq0/01$. The results are presented as the ratio of the experiment to the control. Absolute values and statistical data are presented in Table 3 of the supplementary materials.

Table 3. Dopamine content and calpain activity in CNS cells after a course of reserpine administration in the dose of 1 mg/kg daily, 4 days. The results are presented as the ratio of the experiment to the control.

		Dopamine	Calpain 1	Calpain 2
Hippocampus	Control	1.98 ± 0.88	1.60 ± 1.77	5.37 ± 1.18
	Experiment	1.72 ± 0.83	2.57 ±0.81	3.94 ± 0.89
	Experiment/Control	0.87 ± 0.8 (p=0.656, t=2.365)	1.61 ± 2.27 (p=0,67, t=3.182)	0.73 ± 0.33 (p=0.2, t=2.447)
Striatum	Control	55.85 ± 51.44	1677.09 ± 330.82	953.87 ± 265.02
	Experiment	89.83 ± 59.39	2475.06 ± 1196.76	754.39 ± 311.54
	Experiment/Control	1.6 ± 2.5 (p=0.42, t=2.447)	1.48 ± 0.0 (p=0.171, t=2.447)	0.79 ± 0.55 (p=0.054, t=2.571)
Midbrain	Control	1.68 ± 0.52	7276.96 ± 482.45	4531.49 ± 1397.59
	Experiment	2.69 ± 0.79	5448.99 ± 1693.05	3098.81 ± 355.41
	Experiment/Control	1.6 ± 0.8 (p=0.026, t=2.365)	0.8 ± 0.3 (p=0.02, t=2.776)	0.7 ± 0.3 (p=0.034, t=2.571)

Table 4. Dopamine content and calpain activity in CNS cells of DAT-KO rats. The results are presented as the ratio of the experiment to the control.

		Dopamine	Calpain 1	Calpain 2
Hippocampus	Control	0.4 ± 0.05	1586.09 ± 226.53	4866.07 ± 1204.69
	Experiment	0.9 ± 0.1	2039.09 ± 274.34	7485.46 ± 270.35
	Experiment/Control	2.3 ± 0.32 (p<0.01, t=2.447)	1.29 ± 0.9 (p=0.47, t=0.77)	1.53 ± 0.1 (p=0.05, t=1.954)
Striatum	Control	64.3 ± 2.7	1606.20 ± 86.43	5554.45 ± 240.81
	Experiment	7.5 ± 1.7	2931.14 ± 364.94	6790.16 ± 179.03
	Experiment/Control	0.11 ± 0.03 (p=0.048, t=2.140)	1.82 ± 0.33 (p=0.05, t=2.128)	1.22 ± 0.09 (p=0.2, t=1.423)
Midbrain	Control	5.1± 0.9	N/A	18.42 ± 5.63
	Experiment	2.8 ± 0.1	N/A	21.12 ± 1.44
	Experiment/Control	0.55 ± 0.02 (p=0.04, t=2.692)	N/A	1.15 ± 0.35 (p=0.39, t=2.447)

The activity of calpain 2 was also higher by 1.53 ± 0.40 (p=0.05) in the hippocampus of DAT-KO rats compared to control rats, as shown in Fig.6. It is worth noting that these DAT-KO rats were not exposed to any additional influences, so their increased or decreased calpain activity is intrinsic to them.

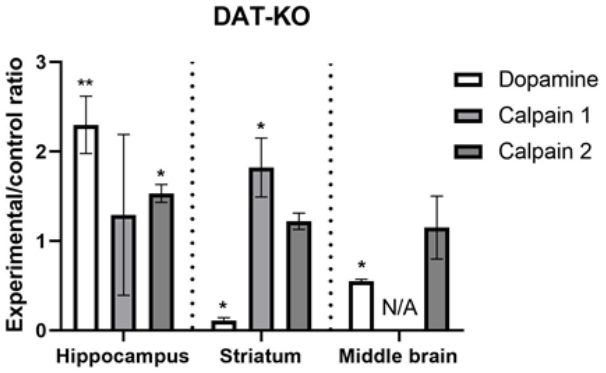


Fig. 5. Dopamine content and calpain activity in CNS cells of DAT-KO rats. * - p<0.05, ** - p≤0/01. The results are presented as the ratio of the experiment to the control. Absolute values and statistical data are presented in Table 4 of the supplementary materials.

4. Discussion

We demonstrated that pharmacological effects on dopamine metabolism in different brain regions affect not only dopamine levels but also the activity of calpain-1 and calpain-2, with region-specific effects. For example, L-DOPA administration increased dopamine levels in the striatum but caused a decrease in dopamine in the hippocampus. In DAT-KO rats, the observed changes were opposite (this is reflected in the graphical abstract).

This phenomenon can be explained by considering the consequences of increased intracellular dopamine (in the L-DOPA administration model) and increased extracellular dopamine (in DAT-KO animals), while taking into account the different densities of dopaminergic terminals and the presence of non-dopaminergic uptake systems in the striatum and hippocampus. The striatum has a high density of dopaminergic terminals; therefore, dopamine synthesized from L-DOPA is efficiently retained within striatal dopaminergic terminals. In contrast, the hippocampus is a region with sparse dopaminergic innervation, and L-DOPA is preferentially converted to norepinephrine, as we have previously demonstrated in our studies [18].

DAT-KO rats represent a model of excessive extracellular dopamine. In the striatum, extracellular dopamine is rapidly metabolized. In the hippocampus, however, dopamine and its metabolites are cleared more slowly than in the striatum due to the absence of an effective system for the utilization of dopamine, which is not a predominant endogenous neurotransmitter in this region [19].

Regarding calpains, L-DOPA administration increased the activity of both calpain-1 and calpain-2 in the striatum compared with the control group. This pattern indicates the simultaneous presence of a neuroprotective effect mediated by calpain-1 activation and a damaging effect mediated by calpain-2 activation. In the hippocampus, L-DOPA reduced calpain-2 activity (Table 1) [14,20]. Considering the possibility of direct activation of calpain-2 by dopamine [21], these findings are consistent with a direct relationship: lower dopamine levels are associated with lower calpain activity. Based on these data, the neuroprotective effect of L-DOPA on striatal neurons could potentially be enhanced by its co-administration with a calpain-2 inhibitor, thereby reducing iatrogenic effects. The selection of a calpain-2 inhibitor with high selectivity remains challenging, but Chagniel et al. investigated the combined administration of L-DOPA and the calpain inhibitor MDL28170 in a rat model of Parkinson's disease induced by 6-hydroxydopamine (6-OHDA). They demonstrated that calpains and Cdk5 in the striatum play a key role in the behavioral and molecular changes associated with L-DOPA therapy. L-DOPA reduced the intensity of Parkinson's disease symptoms in behavioral tests but induced dyskinesia. Intrastriatal infusion of MDL28170, and, to a lesser extent, the Cdk5 inhibitor roscovitine, reduced the severity and amplitude of abnormal involuntary movements without affecting the antiparkinsonian efficacy of L-DOPA. At the molecular level, both inhibitors affected striatal signaling pathways, including ERK1/2 and dynamin phosphorylation. In addition, combined treatment with L-DOPA and MDL28170 increased tyrosine hydroxylase levels in the striatum without affecting the number of dopaminergic neurons in the substantia nigra [22]. At the same time, when designing combined therapies involving L-DOPA and calpain inhibitors, it is important to consider that excess dopamine in the striatum can be converted into quinone forms, thereby enhancing oxidative cellular damage [23]. Therefore, protective mechanisms against dopamine quinones should also be considered, as these reactive species may contribute to calpain-2 activation.

In the AMPT-treated model, we found that 3 hours after AMPT administration, dopamine levels decreased significantly, accompanied by a slight increase in calpain 1 activity (Table 2). It is not possible to compare the obtained results with the literature data, since the activity of calpains upon administration of AMPT has not been analyzed previously. Regarding the effects on the dopaminergic system, it is known that, despite a decrease in the DA concentration, halothane caused an increase in the content of DA metabolites in rats pre-treated with apomorphine. Reserpine completely, and AMPT partially counteracted the increase in the content of DA metabolites caused by halothane anesthesia [24]. AMPT is known to inhibit dopamine formation in the cytosol of dopaminergic neurons and is therefore used to study the role of cytosolic DA pools. In a study by Shu Watanabe, the effects of local administration of AMPT on DA efflux from the nucleus accumbens and dorsal striatum were analyzed using *in vivo* brain microdialysis in non-anesthetized rats. Administration of AMPT (100 μM, 4 h) into the nucleus accumbens reduced DA efflux in the accumbens to 30% from the initial/base level. However, when injected into the dorsal striatum, it reduced DA efflux in the striatum to 60% from the base level [25]. At first glance, these data suggest that the amount of DA available from AMPT-sensitive neurons is greater in the nucleus accumbens than in the striatum. However, this is not the case, since the reduction in DA efflux in the nucleus accumbens and striatum induced by systemic administration of AMPT (250 mg/kg i.p.) was identical. These results indicate that local infusion of AMPT is a valuable tool for understanding the role of AMPT-sensitive neurons in a specific brain region, but it cannot be used to compare effects in different brain structures, since a fixed dose of AMPT affects the nucleus

accumbens and striatum differently.

We have shown that after 4 days of reserpine administration, protease activity in the midbrain decreased: calpain 1 by 1.3 ± 0.3 times, calpain 2 by 1.5 ± 0.3 times. Another study showed that systemic administration of low doses of reserpine (up to 1 mg/kg) to rats is a valuable experimental model for studying non-motor symptoms of PD. The effects of reserpine (1 mg/kg, subcutaneously) on short-term memory and locomotion, as well as the levels of dopamine, serotonin and its metabolites in the striatum, hippocampus and prefrontal cortex have been investigated. Reserpine administration resulted in memory impairment as well as an increase in striatal dopamine levels without changes in spontaneous locomotor activity of rats 72 h after administration [26]. Reserpine is a drug for lowering blood pressure, but in research, it is mainly used as a drug that reduces basal dopamine levels. Dluzen and Liu investigated the effects of L-dopa on dopamine secretion *in vitro* from superfused fragments of male rat striatum pretreated with reserpine [27]. Male rats were injected with reserpine (5 mg/kg) 24 h before sacrifice and immediate striatal superfusion. Dopamine release was stimulated by acute 10-min and continuous 60-min striatal infusions of L-dopa (5 μM). The reduction in monoamine storage capacity achieved by reserpine significantly reduced the amount of basal dopamine released from superfused male rat striatal fragments. Although basal release rates were significantly reduced, the amount of dopamine secreted from striatal neurons in response to *in vitro* L-dopa infusions (10 or 60-min infusions) was equivalent for reserpine-treated animals.

Calpain activity in DAT-KO rats has not been previously examined. In the striatum of DAT-KO rats, dopamine is rapidly metabolized and cannot be taken up into cells due to the absence of the dopamine transporter. In the extracellular space, dopamine and/or its metabolites can activate an extracellular form of calpain-2, as we have previously shown [21], which may disrupt normal CNS regulation. In contrast, the hippocampus expresses a high density of norepinephrine transporters capable of dopamine uptake. In addition, extracellular dopamine that is not packaged into synaptic vesicles may preferentially activate calpain-1.

We have demonstrated that pharmacological interventions in dopamine metabolism in different brain structures change not only the level of dopamine, but also the activity of calpain 1 and calpain 2. Furthermore, for each of the models we used (levodopa, reserpine, and AMPT administration; DAT-KO rats), we observed region-specific changes in dopamine levels and calpain activity. This suggests that it is important to target the delivery of drugs that regulate the dopaminergic system to specific regions of the brain. To reduce the risk of unwanted side effects caused by increased calpain activity, we recommend combining drugs that regulate dopamine levels with calpain inhibitors.

To improve the conclusions of this work, it is necessary to conduct additional studies to identify the influence of the sex and age of the animals, as well as the dose and route of administration of the dopamine system modulators used, on the analyzed parameters.

Funding sources

This work was supported by the Federal State Budgetary Scientific Institution "Institute of Experimental Medicine", St. Petersburg, Russia [project numbers FGWG–2022–0008 (initial experiments), FGWG-2025-0016 (final experiments)]

Acknowledgments

We thank Prof. Raul R. Gainetdinov (Institute of Translational Biomedicine, St. Petersburg State University, Russia) for providing DAT-KO rats.

CRediT authorship contribution statement

Nina Pestereva: Conceptualization, Writing – original draft. Dmitrii Traktirov: Resources, Software. Viktoria Burdinskaya: Investigation. Ekaterina Kulikova: Investigation. Timur Akbarov: Writing – review and editing. Zamira Muruzheva: Methodology, Validation, Writing – original draft. Marina Karpenko: Project administration, Supervision, Writing – review and editing.

Conflict of interest (COI)

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical approvals and statements

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) and certified by the local ethics committee of the Institute of Experimental Medicine (Ethics Issue No. 1/20, 2020). The rats were decapitated using a guillotine (OpenScience AE1601, OOO SPC OpenScience, Russia).

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