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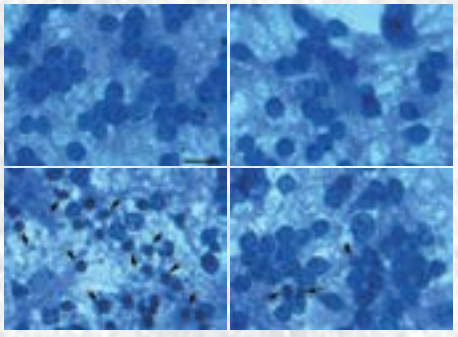

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On the front cover: part of the Figure 2 from the article of E.V. Stelmashook et al. (p. 52).

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В номере:

The Long-Term Course of Chronic Inflammatory Demyelinating Polyneuropathy: a Retrospective Study

Evgeniya A. Melnik, Alina S. Arestova, Irina A. Berdalina, Elena V. Gnedovskaya, Darya A. Grishina, Natalia A. Suponeva, Mikhail A. Piradov

Research Center of Neurology, Moscow, Russia

Abstract

Introduction. *Chronic inflammatory demyelinating polyneuropathy (CIDP) is characterized by long-term progressive or relapsing course, neurological deficit, and disability of varied severity. The course of CIDP after specific therapy and, if necessary, long-term maintenance treatment are to be studied.*

Objective: *To evaluate CIDP clinical and history characteristics over the long-term follow-up (> 5 years), to compare long-term CIDP course in a number of clinical variants and onset types, and to determine clinical predictors of unfavorable CIDP course.*

Materials and methods. *The study included 45 patients diagnosed with CIDP based on EAN/PNS 2021 criteria lasting for 5 or more years. Retrospective collection and analysis of medical records and clinical history were performed. Internationally accepted scales were used to assess neurological deficit (NIS, MRCss), disability (INCAT), and disease activity status (CDAS). The criteria of unfavorable course were developed to evaluate factors affecting CIDP course.*

Results. *Among the patients with CIDP history of >5 years, each third (34%) had no neurological deficit and remained in long-term clinical remission (CDAS 1). The vast majority (90%) responded to first-line therapy in early disease, while only 53% of patients required maintenance treatment in 5 or more years of the onset. With the developed criteria (poor response to glucocorticosteroids (GCS), need for maintenance therapy, and CDAS 3–5), unfavourable CIDP course was detected in 24 (53.3%) participants. Its probability increased in later onset (47 [30; 50] years), the chronic type of onset, and delayed specific therapy. The most significant predictors included low total NIS score at onset (<60 points) and multifocal CIDP.*

Conclusions. *The course of typical CIDP is relatively favorable if timely diagnosed, and pathogenetic treatment initiated. Patients with acute and subacute onset demonstrate the best long-term status. The predictors of unfavourable disease course include mild neurological deficit at onset (NIS total score <60 points) and multifocal CIDP.*

Keywords: *chronic inflammatory demyelinating polyneuropathy; predictors of unfavorable course; typical CIDP; multifocal CIDP; disease activity status; CDAS*

Ethics approval. The study was conducted with the informed consent of the patients. The study protocol was approved by the Ethics Committee of the Research Center of Neurology (Protocol No. 8-4/20, 7 October 2020).

Source of funding. The study was conducted by the Research Center of Neurology on state assignment.

Conflict of interest. The authors declare no apparent or potential conflicts of interest related to the publication of this article.

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Ретроспективный анализ многолетнего течения хронической воспалительной демиелинизирующей полинейропатии

Е.А. Мельник, А.С. Арестова, И.А. Бердалина, Е.В. Гнедовская, Д.А. Гришина, Н.А. Супонева, М.А. Пирадов

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Аннотация

Введение. *Хроническая воспалительная демиелинизирующая полинейропатия (ХВДП) характеризуется многолетним прогрессирующим или рецидивирующим течением, развитием неврологического дефицита и инвалидизации разной степени выраженности. В настоящее время недостаточно изучен характер течения ХВДП в отдалённом катамнезе после проведения первичного курса патогенетической терапии и при необходимости поддерживающего лечения в течение длительного времени.*

Цель *исследования — оценить клинико-анамнестические характеристики течения ХВДП на отдалённых сроках болезни (больше 5 лет), сравнить особенности многолетнего течения ХВДП при разных клинических вариантах и типах дебюта, определить клинические факторы прогноза неблагоприятного течения ХВДП.*

Материалы и методы. *В исследование были включены 45 пациентов с длительностью ХВДП (EAN/PNS 2021) 5 лет и более. Проведён ретроспективный анализ медицинских документов, сбор клинико-анамнестических данных. С помощью общепринятых международных шкал оценивали неврологический дефицит (NIS, MRCss) и степень инвалидизации (INCAT), а также статус активности болезни (CDAS). Для анализа факторов, влияющих на ХВДП, были разработаны критерии «неблагоприятного» течения.*

Результаты. *Каждый третий (34%) пациент со сроком болезни ХВДП более 5 лет не имел неврологического дефицита и находился в стойкой клинической ремиссии (CDAS 1). Подавляющее большинство больных (90%) отвечали на патогенетическую терапию первой линии в первые годы болезни, через 5 и более лет от момента начала заболевания медикаментозное поддержание ремиссии требовалось лишь половине (53%). Согласно разработанным нами критериям неблагоприятное течение (недостаточный ответ на терапию глюкокортикостероидами, необходимость поддерживающих курсов терапии, CDAS 3–5) выявлено у 24 (53,3%) участников. Его вероятность повышалась при более позднем возрасте дебюта (47 [30; 50] лет), хроническом характере дебюта, задержке в начале патогенетической терапии. Наиболее значимыми факторами оказались низкий общий балл NIS в дебюте болезни (< 60 баллов), а также мультифокальный вариант ХВДП.*

Заключение. *Типичная форма ХВДП характеризуется относительно благоприятным течением при условиях своевременной диагностики и начала патогенетической терапии. Наилучший статус в отдалённом катамнезе имеют пациенты с остро-подострым дебютом ХВДП. Факторами прогноза неблагоприятного течения являются невыраженный неврологический дефицит в дебюте (общий балл по NIS < 60) и мультифокальный вариант ХВДП.*

Ключевые слова: *хроническая воспалительная демиелинизирующая полинейропатия; факторы прогноза неблагоприятного течения; типичная ХВДП; мультифокальная ХВДП; статус активности болезни; статус активности болезни*

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Introduction

Chronic inflammatory demyelinating polyneuropathy (CIDP) is a heterogeneous group of treatable chronic immune-mediated polyneuropathies. CIDP is characterized by long-term progressive and/or relapsing course associated with muscle weakness and various sensory disorders, varying from mild and unrestricting daily living or mobility to severe and disabling. As a rule, CIDP patients need long-term first- or second-line specific maintenance therapy as though neither definitive therapeutic regimen nor laboratory markers of disease activity have been established [1–5].

Considering course of CIDP, neurological deficit, and need for specific therapy, K. Gorson et al. introduced the term 'CIDP disease activity status' ('CDAS') and developed simple, clinically usable classification [6].

According to the proposed classification, CIDP can be considered as cured/permanent clinical remission (CDAS 1A, 1B), if the patient's neurological status remains stable for 5 or more years of specific therapy. In progressive or relapsing course, despite immune therapy of any duration, the patient is considered as having unstable active disease (CDAS 5A, 5B, 5C). The authors assessed 106 patients with mean CIDP duration of 6.4 years and demonstrated stable neurological status without any maintenance therapy in 11% of the patients with follow-up of >5 years and unstable active condition without adequate response to therapy in 18% of the patients [6].

Due to complicated underlying pathophysiology, we still question how favorable CIDP course can be in adequate response to specific therapies and which factors might contribute to unfavorable course. Undercovered issues include long-term CIDP, long-term efficacy and tolerability of various therapeutic regimens, and persistent neurological deficit and disability in patients receiving long-term maintenance treatment. Over the past 20 years, only few studies attempted to identify predictors for unfavorable course of CIDP. No uniform approach to selection and evaluation of CIDP patients has led to contradictory conclusions. As a result of the 5-year observation that included 38 patients, S. Kuwabara et al. figured out that the patients with complete remission (26%) more often had subacute onset (4–8 weeks), symmetric symptoms, good response to initial GCS treatment, and nerve conduction abnormalities predominant in the distal nerve terminals [7]. The long-term prognosis of CIDP patients was generally favourable, but 39% of patients still required specific treatments and 13% had severe disability [7]. As a result of the long-term observation that included 60 patients with established CIDP, E. Spina et al. concluded that severe neurological deficit in early disease and later onset are predictors of longer disability regardless of disease duration [8]. As a result of the observation that included 51 patients with CIDP for over 10 years, A. Al-Zuhairy et al. emphasized timely initiation of specific therapy due to revealed relation between the time of therapy initiation and the long-term CIDP prognosis [9].

Therefore, long-term multifocal CIDP (mCIDP) and history of CIDP with acute and subacute onset (A-SA-CIDP) are understudied. Similarly, no Russian experience of CIDP management for over 5 years has been systematically studied and published. Long-term CIDP may indicate whether it is a treatable disease with a good prognosis and when unfavorable course may be suggested.

The study is **aimed** to evaluate CIDP clinical and history characteristics over the long-term follow-up (> 5 years), to compare long-lasting CIDP course with various clinical variants and onset types, and to reveal the clinical predictors of unfavorable CIDP course.

Materials and methods

The study included patients aged >18 years diagnosed with CIDP based on EAN/PNS 2021 criteria lasting for 5 or more years. The 5-year threshold of disease duration was based on the CDAS clinical guidelines [6]. Patients were not included in case of any severe decompensated medical condition or abnormal M gradient secretion (by blood and urine protein electrophoresis plus anti-IgG, anti-IgA, anti-IgM, anti-light chain kappa, and anti-light chain lambda antiserum immunofixation tests).

All the study participants signed informed consent forms for taking part in the study and for personal data processing. The study protocol was approved by the Ethics Committee of the Research Center of Neurology (Protocol No. 8-4/20, 7 October 2020).

At baseline visit, past and present history, neurological examination, and disability assessment were performed. We used internationally accepted scales including Neuropathy Impairment Score (NIS) and Medical Research Council sum score (MRCss) to assess patients' neurological status, and Inflammatory Neuropathy Cause and Treatment (INCAT) to measure their activity limitation [10–15]. Additionally, the medical records were retrospectively analyzed to specify the course of disease and response to specific therapies and to assess patients' neurological status at CIDP onset (by results of examination at the time of diagnosis).

Basing on past history and baseline examination, we specified the following characteristics:
1) clinical variant: typical CIDP (tCIDP) vs mCIDP;
2) chronic onset (CIDP) (symptoms worsening >8 weeks) vs A-SA-CIDP (<8 weeks);
3) relapses (both spontaneously and on therapy) throughout the disease period;
4) progression throughout the disease period.

Considering baseline neurological examination, disease duration, specific therapy duration, and response to therapy, we assessed CIDP activity status (CDAS) [6].

To evaluate factors contributing to CIDP prognosis, we developed **the criteria of unfavorable course**. They include scored CIDP activity status and scored response to specific therapies, taking into account need for first- or second-line maintenance as well as poor response to predominantly used GCS therapy. The criteria are presented in Table 1. The course of CIDP was considered unfavorable if the total score was less than 4. In other words, in stable inactive disease (CDAS 3), with at least 1 of 3 criteria of poor response to specific therapy, or in unstable active disease (CDAS 4/5)

Table 1. The criteria of unfavorable CIDP

Criteria	Value	Score
Poor response to GCS (no improvement on GCS)	No	0
	Yes	1
Need for maintenance treatment (IVIG/GCS/plasmapheresis/GCS + IVIG)	No	0
	Yes	1
Need for the 1st and/or the 2nd line specific therapy at baseline	No	0
	Yes	1
CIDP disease activity status (CDAS)*	1A, 1B	1
	2A, 2B	2
	3A, 3B	3
	4A, 4B	4
	5A, 5B, 5C	5

Note. IVIG, intravenous high-dose human immunoglobulin. *Unfavourable CDAS with follow-up of ≥ 5 years: 3A-B, 4A-B, 5A-C.

CIDP course was considered unfavorable regardless of other criteria.

Statistical analysis was performed using SPSS Statistics 23.0 (IBM, Armonk, NY, USA). Two-sided criteria were used in all cases. The null hypothesis was rejected at $p = 0.05$.

Median and quartiles were used to describe quantitative and ordinal variables whereas frequency and percentages were used to describe categorical variables. Quantitative and ordinal variables in two unrelated groups were compared using the Mann—Whitney test. Categorical variables in two unrelated groups were compared using the Pearson's χ^2 test or the Fisher's exact test (under constraints). Quantitative variables in two unrelated groups were compared using the Wilcoxcon test.

Predictors of unfavorable CIDP were identified using binary logistic regression with sequential Wald selection of predictors. The model included potential predictors selected by comparison of favorable and unfavorable course groups as described above. Thresholds for quantitative predictors were determined by ROC analysis calculating the Youden's index.

Results

Evaluation of long-term CIDP course

The study included 45 patients, of whom 24 (53.3%) women and 21 (46.7%) men, with CIDP duration of 5 or more years. At baseline, the median [Q25%; Q75%] age was 50 [37; 58] years and the median duration of symptomatic disease was 10 [7; 14] years.

The sample included 33 (73.3%) patients with tCIDP, 12 (26.7%) patients with mCIDP, and no patients with other CIDP clinical variants. The disease had ChO in 28 (62.2%) patients and A-SA-CIDP in 17 (37.8%) patients. CIDP progressed in 24 (53.3%) patients and relapsed in 23 (51.1%) patients.

At onset, all the participants had significant neurological deficit (total NIS 56 [35; 94], total MRCss 54 [46; 58]), and disability (total INCAT 3 [2; 5]). In 5 or more years of the onset, these scores improved. At baseline, total NIS was 21 [13; 46] ($p = 0.001$), total MRCss was 60 [54; 60] ($p = 0.008$), and total INCAT was 1 [0; 3] ($p = 0.006$) (the confidence levels were compared to the corresponding onset confidence levels).

Fifteen (33.4%) participants demonstrated persistent clinical remission for ≥5 years without any specific therapy (CDAS 1A-B). Other 6 (13.3%) participants had clinical remission for <5 years without any specific therapy (CDAS 2A-B). Eleven (24.4%) participants had stable neurological status for ≥1 year on specific therapy (CDAS 3B), 5 (11.1%) participants had stable neurological status for 3–12 months on pathogenetic therapy (CDAS 4B). Unstable active disease was documented in 8 participants including 2 (4.4%) patients on no specific therapy (CDAS 5B) and 6 (13.3%) patients on therapy (CDAS 5C).

We compared patients with A-SA-CIDP and CIDP to eva-luate CIDP course (Table 2). At onset, the patients with A-SA-CIDP were younger than those with CIDP without any significant difference ($p = 0.077$). Median onset-to-diagnosis time was 1 [1; 3] month in A-SA-CIDP and10 [4; 66] months in CIDP ($p < 0.001$), which may be related to slow worsening of symptoms

Table 2. Clinical and history characteristics of CIDP patients with various onset types

Characteristics	A-SA-CIDP (< 8 weeks)	CIDP (> 8 weeks)	*p*
Number of participants, *n*	17	28	
Sex, *n* (%):			
male	9 (52.9%)	12 (42,9%)	0.552
female	8 (47.1%)	16 (57,1%)	
Age at onset, years; Me [Q25%; Q75%]	26 [18; 43]	42 [29; 50]	0.077
Disease duration, years; Me [Q25%; Q75%]	10 [8; 13]	10 [7; 15]	0.823
Onset-to-therapy time, months; Me [Q25%; Q75%]	1 [1; 2]	10 [4; 70]	< 0.001
CIDP variant, *n* (%):			
typical	15 (88.2%)	18 (64,3%)	0.096
multifocal	2 (11.8%)	10 (35,7%)	
Progressive course, *n* (%)	3 (17.6%)	21 (75,0%)	< 0.001
Non-progressive course, *n* (%)	14 (82.4%)	7 (25,0%)	
Relapsing course, *n* (%)	9 (52.9%)	14 (50,0%)	1.000
Non-relapsing course, *n* (%)	8 (47.1%)	14 (50,0%)	
NIS, total score, Me [Q25%; Q75%]			
at onset	94 [76; 97]	41 [24; 55]	< 0.001
at baseline	14 [6; 20]	30,5 [20; 66]	< 0.001
INCAT, total score, Me [Q25%; Q75%]			
at onset	5 [3; 5]	2 [2; 3]	< 0.001
at baseline	0 [0; 1]	2 [0; 4]	0.003

in CIDP. Median onset-to-therapy time was 1 [1; 2] month in A-SA-CIDP and 10 [4; 70] months in CIDP ($p < 0.0001$). Participants with CIDP had progressive CIDP more often than those with A-SA-CIDP (75% vs 17,6%, $p < 0.001$).

Initially, the patients with CIDP had more severe neurological deficit, i.e. higher NIS ($p < 0.001$) and higher MRCss ($p < 0.001$), and more significant disability, i.e. higher INCAT ($p < 0.001$). However, at baseline (in 5 or more years of onset) the participants with A-SA-CIDP demonstrated milder NIS ($p < 0.001$) and MRCss ($p = 0.012$) neurological deficit and slight INCAT disability ($p = 0.003$).

We compared the patients with tCIDP and mCIDP to evaluate the CIDP course in different clinical variants (Table 3). The patients with mCIDP were older than those with tCIDP though non-significantly ($p = 0.083$). Median worsening time was 3 [1; 6] months in tCIDP and 66 [7; 132] months in mCIDP ($p = 0.003$), which affected CIDP diagnosis establishment and specific therapy initiation, with mean onset-to-therapy time of 3 [2; 9] months in tCIDP and 66 [8; 108] months in mCIDP ($p = 0.011$).

At onset, tCIDP manifested with symmetric symptoms while mCIDP had asymmetric ones ($p = 0.002$). In early disease, lower limbs were affected more often in the patients with tCIDP including both muscle weakness (87.9% vs 33.3% in the patients with mCIDP; $p = 0.001$) and sensory disorders (72.7% vs 33.3% in the patients with mCIDP; $p = 0.034$). At onset, NIS, MRcss, and INCAT scores in the pa-

Table 3. Clinical and history characteristics of patients with CIDP variants

Characteristics	tCIDP	mCIDP	*p*
Number of participants, *n*	33	12	
Sex, *n* (%):			
male	14 (42.4%)	7 (58.3%)	0.501
female; *n* (%)	19 (57.6%)	5 (41.7%)	
Age at onset, years; Me [Q25%; Q75%]	30 [18; 50]	43 [40; 49]	0.083
Disease duration, years; Me [Q25%; Q75%]	10 [7; 15]	8 [6; 11]	0.151
Duration of symptoms worsening, months; Me [Q25%; Q75%]	3 [1; 6]	66 [7; 132]	0.003
Onset-to-therapy time, months; Me [Q25%; Q75%]	3 [1; 6]	70 [12; 132]	0.011
Onset type, *n* (%):			
acute-subacute (< 8 weeks)	15 (45.5%)	2 (16.7%)	0.096
chronic (> 8 weeks)	18 (54.5%)	10 (83.3%)	
Progressive course, *n* (%)	15 (45.5%)	9 (75.0%)	0.101
Non-progressive course, *n* (%)	18 (54.5%)	3 (25.0%)	
Relapsing course, *n* (%)	18 (54.5%)	5 (41.7%)	0.514
Non-relapsing course, *n* (%)	15 (45.5%)	7 (58.3%)	
NIS, total score; Me [Q25%; Q75%]			
at onset	76 [43; 96]	22 [12; 53]	< 0.001
at follow-up	20 [10; 28]	63 [20; 81]	0.008
INCAT, total score; Me [Q25%; Q75%]			
at onset	3 [2; 5]	2 [1; 2]	0.001
at follow-up	0 [0; 2]	4 [2; 5]	0.001
Symptoms at onset, *n* (%):			
motor (UL)	22 (66.7%)	8 (66.7%)	1.000
motor (LL)	29 (87.9%)	4 (33.3%)	0.001
sensory (UL)	20 (60.6%)	8 (66.7%)	1.000
sensory (LL)	24 (72.7%)	4 (33.3%)	0.034
symmetric	28 (84.8%)	4 (33.3%)	0.002
asymmetric	5 (15.2%)	8 (66.7%)	
Symptoms in the follow-up period, *n* (%):			
motor (UL)	13 (39.4%)	11 (91.7%)	0.002
motor (LL)	18 (54.5%)	10 (83.3%)	0.096
sensory (UL))	14 (42.4%)	10 (83.3%)	0.020
sensory (LL)	22 (66.7%)	8 (66.7%)	1.000
symmetric	23 (92.0%)	3 (25.0%)	< 0.001
asymmetric	2 (8.0%)	9 (75.0%)	

Note. UL, upper limbs; LL, lower limbs.

tients with tCIDP also indicated more severe disease than in those with mCIDP ($p < 0.001$, $p = 0.002$, and $p = 0.001$, respectively).

At baseline, 15 (45.5%) patients with tCDIP showed no muscle weakness, while 11 (91.7%) patients with mCIDP still had limb pareses. The patients with tCIDP still had symmetric signs more often, while the patients with mCIDP typically had asymmetric ones ($p < 0.001$). At baseline, upper limbs were affected significantly more often in the patients with mCIDP including both muscle weakness (91.7% vs 39.4% in the patients with tCIDP; $p = 0.002$) and sensory disorders (82.3% vs 42.4% in the patients with tCIDP; $p = 0.020$). Despite more severe tCIDP onset, at baseline the tCDIP patients' NIS, MRCss, and INCAT scores indicated milder disorders than those scores in mCDIP patients ($p = 0.008$, $p = 0.004$, and $p = 0.001$, respectively), which suggests that tCDIP is more treatable.

Table 4 outlines evaluation of specific therapies in patients with CIDP variants. Interestingly, the patients with mCIDP significantly more likely needed specific therapy to maintain remission than those with tCIDP in long-term follow-up (83.3% vs 42.4%, $p = 0.020$), while maintenance treatment was necessary in 4 (23.5%) patients with A-SA-CIDP and in 20 (71.4%) patients with CIDP ($p = 0.002$). After GCS therapy, each third

Table 4. Evaluation of specific therapy based on CIDP variants

Therapeutic options	tCIDP	mCIDP	*p*
Specific therapy, *n* (%)	32 (97.0%)	9 (75.0%)	0.052
Overall response to therapy, *n* (% of the patients received)	31 (96.9%)	7 (77.8%)	0.044
Need for follow-up maintenance treatment at baseline, *n* (%)	14 (42.4%)	10 (83.3%)	0.020
GCS therapy; *n* (%)	31 (93.9%)	9 (75.0%)	0.109
Response to GCS, *n* (% of the patients received)	23 (74.2%)	2 (22.2%)	0.010
Need for follow-up GCS maintenance treatment, *n* (% of the patients received)	12 (38.7%)	3 (33.3%)	1.000
Carrying out plasmapheresis; *n* (%)	23 (69.7%)	5 (41.7%)	0.163
Response to plasmapheresis, *n* (% of the patients received)	16 (69.6%)	3 (60.0%)	0.586
IVIG therapy, *n* (%)	17 (51.5%)	7 (58.3%)	0.746
Response to IVIG, *n* (% of the patients received)	14 (82.4%)	6 (85.7%)	1.000
Need for follow-up IVIG maintenance treatment, *n* (% of the patients received)	9 (52.9%)	7 (100%)	0.054
Need for follow-up IVIG + GCS maintenance treatment, *n* (% of the patients received)	6 (37.5%)	1 (14.3%)	0.366
Immunosuppression, *n* (% of the patients received)	8 (24.2%)	3 (25.0%)	1.000
Immunosuppression options *n* (% of the patients received):			
azathioprine	5 (62.5%)	1(33.3%)	0.133
cyclophosphamide	1 (12.5%)	0 (0%)	
rituximab + cyclophosphamide	2 (25.0%)	0 (0%)	
rituximab + azathioprine	0 (0%)	2 (66.7%)	
Response to immunosuppression, *n* (% of the patients received)	2 (25.0%)	0 (0%)	0.206

patient (38.7% of the patients with tCIDP, 33.3% of the patients with mCIDP) needed GCS to maintain remission, while only 2 (13.3%) patients with A-SA-CIDP needed GCS after primary therapy.

IVIG was used as primary specific therapy (typically in GCS poor effect and GCS side effects). All the patients with mCIDP and 9 (59.2%) patients with tCIDP needed IVIG maintenance after primary therapy. Six (37.5%) participants with tCIDP and 1 (14.3%) participant with mCIDP needed GCS plus IVIG combination as maintenance treatment.

Immunosuppression was initiated in 8 (24.2%) patients with tCIDP and 3 (25.0%) patients with mCIDP due to poor response to first-line therapy. Immunosuppression had positive response in 2 (25.0%) patients with tCIDP and no patients with mCIDP.

Clinical predictors of unfavorable CIDP course

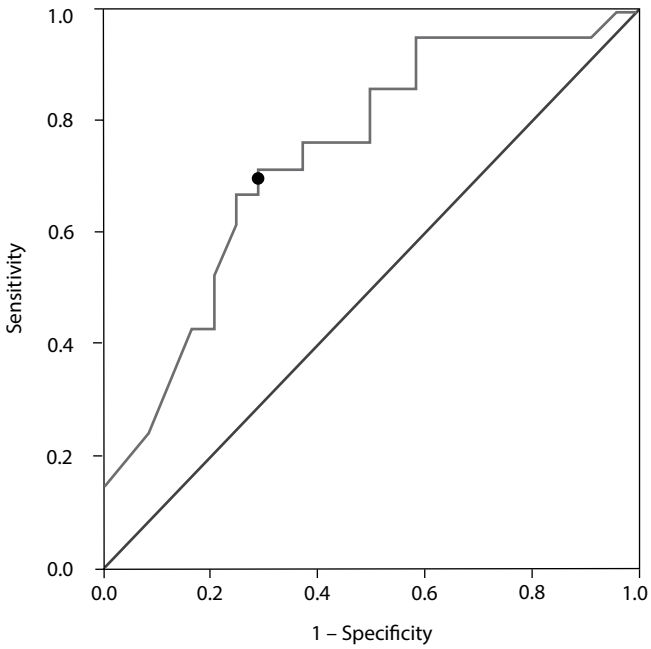
According to our own criteria, at baseline, unfavorable CIDP course was observed in 24 (52.3%) participants, while 21 (46.7%) participants had favorable CIDP course. Table 5 outlines clinical and history characteristics of the patients with favorable or unfavorable CIDP course.

CIDP manifested at the age of 47 [30; 50] in the participants with unfavorable CIDP and at an earlier age of 30 [19; 40] in the participants with favorable CIDP ($p = 0.049$). Unfavorable course was aslo more typical for ChO (83.3% vs 38,1%; $p = 0.002$) and tCIDP (41.7% vs 9.5%; $p = 0.020$).

The patients with unfavorable CIDP course had less prominent neurological deficit at onset. Particularly,

Table 5. Clinical and history characteristics of patients with favorable or unfavorable CIDP course

Characteristics	Unfavourable course	Favourable course	*p*
Number of participants, *n*	24	21	
Sex, *n* (%):			
male	11 (45.8%)	10 (47.6%)	1.000
female	13 (54.2%)	11 (52.4%)	
Age at disease onset, years; Me [Q25%; Q75%]	47 [30; 50]	30 [19; 40]	0.049
Period from onset to initiation of therapy, months; Me [Q25%; Q75%]	12 [2; 120]	2 [1; 3]	0.002
CIDP variant, *n* (%):			
tCIDP	14 (58.3%)	19 (90.5%)	0.020
mCIDP	10 (41.7%)	2 (9.5%)	
Type of disease onset, *n* (%):			
acute-subacute (< 8 weeks)	4 (16.7%)	13 (61.9%)	0.002
chronic	20 (83.3%)	8 (38.1%)	
NIS in onset, total score, Me [Q25%; Q75%]	44 [24; 71]	78 [50; 96]	0.006
Carrying out GCS treatment, *n* (%)	23 (95.8%)	17 (81.0%)	0.169
Carrying out plasmapheresis, *n* (%)	17 (70.8%)	11 (52.4%)	0.233
Carrying out IVIg, *n* (%)	19 (79.2%)	5 (23.8%)	< 0.001
Carrying out immunosuppressant therapy, *n* (%)	8 (33.3%)	3 (14.3%)	0,177



The ROC curve for NIS total score at the disease onset.

median NIS total score was 44 [24; 71] in the participants with unfavorable CIDP course and 78 [50; 96] in the participants with favorable CIDP course ($p = 0.006$). However, at enrollment to the follow-up study, neurological deficit became more prominent in the participants with unfavorable CIDP course. Median NIS total score was 55 [24; 74] in the participants with unfavorable CIDP course and 12 [8; 21] in the participants with favorable CIDP course ($p < 0.001$).

Onset-to-therapy time in the participants with unfavorable CIDP course was 12 [2; 120] months vs 2 [1; 3] months ($p = 0.002$).

We compared the patients with favorable and unfavorable CIDP course and selected a number of predictors including onset age, onset type, onset-to-therapy type, onset NIS, and CIDP clinical variant.

We analyzed the model including the above factors and determined the NIS total score at onset and CIDP clinical variant as predictors of CIDP course. Subsequently, unfavorable course was more probable in mCIDP and, unexpectedly, a lower total NIS score (i.e. milder neurological deficit) at onset.

In one of the CIDP clinical variants, calculation of the significance level and the odds ratio was limited by the small sample. However, the predictive model was reliable ($p < 0.001$ for the model, R2N = 0.456, $P = 0.945$ for the Hosmer–Lemeshow test).

The threshold was determined for the NIS total score at the onset by ROC analysis (see Figure). The AUC [95% CI] was 0.739 [0.593, 0.885]. The Youden's optimum threshold was determined as 60 (probability of unfavorable CIDP course increases with NIS total score at the onset < 60). Sensitivity was 71.4%. Specificity was 70.8%.

Discussion

We performed retrospective analysis of clinical and history data in the sufficient sample of patients with CIDP duration of over 5 years. CDAS indicated clinical remission in 33.4% of the participants without any specific therapy during 5 or more years (CDAS 1A-1B), which demonstrates the possibility of stabilization of neurological status and maintenance treatment withdrawal in long-term follow-up. Nevertheless, 13.3% of the patients had unstable active disease with poor response to therapy.

The sample is distinguished by the patients with both A-SA-CIDP and CIDP as well as both tCIDP and mCIDP included to evaluate the contribution of onset types and clinical variants.

We conclude that A-SA-CIDP was typical for younger patients as compared to CIDP, which corresponds to G. Liberatore's et al. results [16]. At onset, the A-SA-CIDP participants had more significant neurological deficit (NIS 94 [76; 97]) and disability (INCAT 5 [3; 5]). Thus, specific therapy was initiated in most patients with A-SA-CIDP in one month of onset despite incorrect the diagnosis of acute inflammatory demyelinating polyneuropathy. Simultaneously, median onset-to-therapy time was 10 [4; 70] months, i.e. therapy was significantly delayed. The above might be the reason why the A-SA-CIDP patients had milder neurological deficit (NIS 14 [6; 20]) and minimal disability (INCAT 0 [0; 1]) in 5 or more years. S. Kuwabara's et al. results correspond to our results. However, G. Liberatore et al. note less favorable course in A-SA-CIDP patients [7, 16], which may result from late diagnosis of acute CIDP and prolonged management of these patients as Guillain–Barré syndrome (without any GCS therapy).

Our sample included 73% of the participants with tCDIP and 27% of the participants with mCDIP, which corresponds to the M. Mahdi-Roger et al. results [17]. Simultaneously, P. Doneddu et al. observed mCIDP in 4% of the patients [4]. Noteworthily, we were not able to assess IgG4-antibodies (neurofascin 155, contactin 1, contactin-associated protein, and neurofascin 140/186 isoforms) and to establish nodopathies that do not comply with EAN/PNS202 CIDP criteria due to clinical phenotypes, disease courses, and first-line therapy resistance [3, 18, 19]. Nevertheless, most A-SA-CIDP study participants responded to GCS therapy and had slight neurological deficit in long-term follow-up, which is not typical for autoimmune nodopathies.

Therefore, we suppose that the study did not include any patients with autoimmune nodopathies.

In the study, A-SA-CIDP was more common in tCIDP though it was observed in 17% of the patients with mCIDP that typically progresses slowly [20]. In most tCIDP patients, symptoms worsened within six months, and we could establish diagnosis within a year in vast majority of the participants (88%). At onset, the patients with tCIDP had more severe neurological deficit (NIS 76 [43; 96]) and disability (INCAT 3 [2; 5]) as compared to mCIDP. Most patients with tCIDP showed symmetric motor and sensory disorders in the upper and mostly lower limbs, which corresponds to the presumable clinical conception of the disease and its clinical criteria.

When diagnosed, 97% of the participants with tCIDP were recommended specific therapy, usually GCS. Half of the participants with tCIDP received IVIG (often combined with GCS). A quarter of the patients received cytostatics in poor response to first-line therapy. Up to 97% of the participants with tCIDP responded to the specific therapy whereas less than half (42%) of the patients needed maintenance treatment in long term.

Analysis of tCIDP course showed that symptoms remained symmetric, mostly in the lower limbs, in long-term follow-up. The patients further demonstrated less severe neurological deficit (NIS 20 [10; 28]) and disability (INCAT 0 [0; 2]) as compared to mCIDP. Moreover, the patients with tCIDP had less severe neurological deficit and disability in 5 or more years than at onset, which indicates possible recovery of motor function and improvement of functional activity in timely specific therapy and generally confirms that CIDP is a treatable disease with favorable course.

In our study the mCIDP participants had typically later onset, quite mild neurological deficit (NIS total score 22 [12; 53]) and mild disability (INCAT 2 [1; 2]) at onset. In this subpopulation, the disease often (66.7%) manifested asymmetrically, with muscle weakness and sensory disorders (mostly in the upper limbs), which is known to be typical for mCIDP rather than for tCIDP [21]. Slow symptomatic progression (median 66 [7; 132] months) increased onset-to-diagnosis and therapy time. In 57% of the patients, mCIDP was diagnosed in 3 or more years of onset (66 [8; 108] months vs 3 [2; 9) months in tCIDP; $P = .011$).

Seventy-five percent of the participants with mCIDP received specific therapy, while 25% of patients with milder neurological deficit expected to receive IVIG. Only 22% of the patients with mCIDP responded to GCS therapy. IVIG therapy was initiated in 58% of the patients, with response observed in 86% of them, which confirms a better response to IVIG in comparison with response to GCS in mCIDP [20]. In the long term, 83% of the participants with mCIDP, i.e. twice as many as those with tCIDP (42.4%), needed maintenance treatment to achieve remission ($p = 0.020$). Therefore, mCIDP is evidently more difficult to manage than tCIDP.

In long-term follow-up, the patients with mCIDP still demonstrated asymmetric symptoms (mostly in the upper limbs). Thus, the clinical manifestations did not transform to symmetric pattern that would be typical for tCIDP, which was probably related to different pathophysiological mechanisms [18, 22]. At the moment of retrospective analysis (≥5 years of the disease onset), the participants with mCIDP had significantly more severe neurological deficit (NIS 63 [20; 81]) and disability (INCAT 4 [2; 5]) as compared to onset. CDAS 5 in 50% of the patients with mCIDP indicated unstable active disease. Supposedly, specific therapy can only be used for stabilizing disease in most patients with mCIDP. Therefore, we have detailed information on long-term disease course and sufficient evidence to state that, despite specific therapies, mCIDP should not be considered as a quite favorable type, especially with progression of neurological deficit and worsening of disability.

Our results correspond to those of G. Fargeot et al. who emphasized differentiation of mCIDP from other variants to predict therapeutic response that is usually worse than that in tCIDP. They also specify mCIDP features we note including poor effectiveness of GCS and plasmapheresis, IVIG dependence, and a less favorable prognosis in long-term disease [20].

With detailed information on the course of CIDP variants, we made effort to study the predictors of unfavorable course. Basing on our experience and comparisons, we proposed the following criteria of unfavorable course: poor response to GCS therapy; need for maintenance treatment; CDAS 3–5 in long-term follow-up. In accordance with our results, unfavorable CIDP course is more probable in quite mild neurological deficit (NIS total score <60) at onset with another negative predictor being mCIDP. In the literature, the predictors of unfavorable course include late onset, slow progression, asymmetric symptoms, and delayed therapy initiation. Conversely, early onset and A-SA-CIDP, symmetric symptoms, severe neurological deficit at onset, relapsing disease, timely initiation of specific therapy, and adequate response to therapy are considered as positive predictors [7–9, 16, 23]. Our results correspond to the earlier publications on CIDP predictors. Late onset, slow progression, asymmetric symptoms, and longer onset-to-therapy time are typical for mCIDP. Association of low onset NIS score with unfavorable prognosis may be related to the fact that each third patient had mCIDP, with mild onset neurological deficit, typically in the upper limbs, and slow progression. Additionally, we in-

cluded no patients with sensory CIDP, i.e. those with mild deficit and more favorable course.

Conclusion

Therefore, favorable course was typical for tCIDP, as 90% of the patients demonstrated positive response to first-line specific therapy at onset, and 34% of the patients had no neurological deficit with persistent clinical remission in 5 or more years of the disease onset. In long-term follow-up (> 5 years), the patients with tCIDP had less significant neurological deficit and disability than during the first years and only 53% of patients needed maintenance treatment to achieve remission. Unfavorable course is more probable in milder neurological deficit at onset (NIS <60) and mCIDP, which is often associated with later diagnosis and specific therapy initiation. Over time, symptoms stay asymmetric and the upper limbs are more involved in mCIDP with more severe progression of neurological and functional deficits. A-SA-CIDP has typically more favorable course with less significant neurological deficit and the need for maintenance treatment in 23.5% of the patients in long-term follow-up [24].

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Mood Disorders After COVID-19

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Abstract

Introduction. *The COVID-19 pandemic has led to a high prevalence of post-COVID-19 syndrome (PCS), with mood disorders being the most common manifestations.*
Objective: *To study the prevalence of PCS-associated mood disorders and their features.*
Materials and methods. *We examined patients after COVID-19 (n = 91; age: 24–84 years; median time to recovery: 7 months) using the following tools: the BDI and HADS (screening for anxiety and depression); the Starkstein Apathy Scale; FIS and FSS (fatigue assessment); the MoCA, MMSE, and FAB (cognitive assessment); the FIRST, ESS, PSQI, and ISI (sleep disorders evaluation); the EQ5D (quality of life measurement). We designed a special questionnaire to collect data related to a history of COVID-19 and patients' condition after discharge. In addition, we analyzed electronic medical records and discharge summaries and performed neurological examination.*
Results. *Of all the examined patients, 65 (71.4%) participants had signs and symptoms of PCS. Mood disorders were observed in 33 (50.8%) cases, with apathy (78.7%), anxiety (66.7%), and fatigue (60.6%) being the most common. Depressive disorders were found in 12 (36.3%) patients. Cognitive functions were impaired in 7 (21.2%) patients; sleep disorders were observed in 16 (48.5%) cases. We found a positive correlation between depressive disorders and fatigue based on the BDI, FIS, and FSS scores (r_S = 0.711; r_S = 0.453), depressive disorders and anxiety (r_S = 0.366), fatigue and apathy (r_S = 0.350). Anxiety increased the risk of sleep disorders (r_S = 0.683). Quality of life has been shown to decrease in patients with mood disorders due to the negative effect of long-term fatigue and depressive disorders.*
Conclusions. *There is a close connection between different types of mood disorders that develop after COVID-19 and exacerbate symptoms of each other. Early diagnosis and treatment of these disorders can improve patients' quality of life and preserve their ability to work.*

Keywords: *COVID-19; post-COVID-19 syndrome; depression; apathy; anxiety; fatigue*

Ethics approval. The study was conducted with the informed consent of the patients. The research protocol was approved by the Ethics Committee of Almazov National Medical Research Centre (protocol No. 0212-22, 26 December 2022).

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Аффективные нарушения у пациентов, перенёсших COVID-19

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Аннотация

Введение. *Пандемия коронавирусной инфекции (COVID-19) привела к высокой распространённости постковидного синдрома (ПКС), частым проявлением которого являются аффективные нарушения.*
Цель *исследования — изучение встречаемости аффективных нарушений в рамках ПКС и их особенностей.*
Материалы и методы. *Обследованы пациенты, перенёсшие COVID-19 (n = 91; возраст 24–84 года; медиана выздоровления — 7 мес). Использовались опросники: BDI, HADS (выявление тревоги и депрессии); шкала апатии Starskein; FIS, FSS (оценка усталости); MoCA, MMSE, FAB (оценка когнитивных функций); FIRST, ESS, PSQI, ISI (выявление нарушений сна); EQ5D (оценка качества жизни (КЖ)). Сбор анамнеза заболевания COVID-19, состояния пациентов после выписки проводили с помощью специально разработанного опросника. Дополнительно анализировали электронные истории болезней, выписные эпикризы, выполняли неврологический осмотр.*
Результаты. *В исследуемой группе 65 (71,4%) пациентов имели признаки постковидного синдрома. Аффективные нарушения встречались в 33 (50,8%) случаях, наиболее частые из них: апатия (78,7%), тревожность (66,7%), усталость (60,6%). Депрессивные расстройства выявлены у 12 (36,3%) пациентов. У 7 (21,2%) пациентов снизились когнитивные функции. В 16 (48,5%) случаях наблюдались расстройства сна. Выявлена прямая взаимосвязь между депрессивными расстройствами и усталостью, согласно данным BDI, FIS и FSS (r_S = 0,711; r_S = 0,453), депрессивными расстройствами*

и тревожностью (r_S = 0,366), усталостью и апатией (r_S = 0,350). Наличие тревожности повышало риск развития сомнологических расстройств (r_S = 0,683). Выявлено, что при наличии аффективных нарушений снижается КЖ вследствие негативного влияния длительно сохраняющейся усталости и развития депрессивных расстройств.

Заключение. *Разные виды аффективных нарушений, развивающихся после перенесённого COVID-19, тесно связаны между собой, усугубляя проявления друг друга. Раннее выявление и лечение таких расстройств позволит улучшить КЖ и сохранить трудоспособность пациентов.*

Ключевые слова: *COVID-19; постковидный синдром; депрессия; апатия; тревожность; усталость*

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Introduction

The COVID-19 pandemic has led to a high prevalence of post-COVID-19 syndrome (PCS) that physicians of various fields currently deal with. This condition has 2 phases: (1) subacute symptomatic phase with symptoms present from 4 to 12 weeks after COVID-19 and (2) chronic phase with symptoms persisting beyond 12 weeks and not attributable to an alternative diagnosis [1–3]. The prevalence of PCS accounts for 10–65% and reaches 85% in patients hospitalized for acute COVID-19 [3, 4].

Neurological disorders can occur during the first days of the disease. A USA study conducted in 2020 included 509 patients hospitalized with COVID-19 and found that 82.3% of the participants had neurological disorders at any time during the disease course. The most common disorders were myalgia, headache, encephalopathy, dizziness, dysgeusia, and anosmia [5]. These symptoms are referred to as neurological manifestations of post-acute sequelae of SARS-CoV-2 infection (neuro-PASC) [6]. The most common neuro-PASC include memory and attention disorders, signs of depression, apathy, sleep disorders, fatigue, myalgia, headache, and dizziness [7–10]. Apart from individual post-COVID-19 neurological symptoms, some patients develop more serious neurological complications during or after COVID-19: stroke, seizures, neuromuscular disorders and demyelinating diseases such as myasthenia gravis and Guillain-Barré syndrome, etc. [11–14].

The pathogenesis of PCS has not been entirely investigated despite its high prevalence [2]. Neurotropism of SARS-CoV-2 is thought to be linked to its high affinity to receptors of angiotensin-converting enzyme 2 (ACE2) that is expressed not only on type II pneumocytes but also in neurons and glial cells [11]. Moreover, binding of SARS-CoV-2 to ACE2 receptors in the vascular endothelium can cause endotheliitis, coagulopathy, arterial and venous thromboses resulting in such complications as ischemic strokes, cerebral venous thrombosis, intracerebral or subarachnoid hemorrhage [15]. Mood disorders are hypothesized to develop during or after COVID-19 because neuropsychological disorders may be caused by GABAergic dysfunction due to COVID-19-associated inflammation [16]. The literature shows that newly developed depression may be triggered by cytokine release, e.g., interleukin-6 (IL-6), during acute COVID-19, and it resolves as cytokines return to normal levels, regardless of antidepressant treatment. This suggests that medications that lower cytokine activity can reduce the odds of mood disorders after COVID-19; however, further research is required to better understand this process [17].

Our objective is to study the prevalence of PCS-associated mood disorders and their features.

Materials and methods

Our study involved 91 patients (38 men and 53 women) aged 24–84 years (mean age: 58.7 years). COVID-19 was confirmed by PCR tests. Seventy-one (78%) COVID-19 patients were admitted to the rehabilitation clinic of Almazov National Medical Research Centre that functioned as an infectious disease hospital in the summer of 2021. The median time to recovery was

7 months. The study was conducted with the informed consent of the patients. The research protocol was approved by the Ethics Committee of Almazov National Medical Research Centre (protocol No. 0212-22, 26 December 2022).

We performed clinical neurological examination in the outpatient setting.

We designed a questionnaire to collect data related to a history of COVID-19 and patients' condition after discharge. This questionnaire includes several sections that assess history of acute COVID-19 and condition after discharge, chronic diseases, vaccination, and pre-existing cognitive, mood, and/or sleep disorders.

Cognitive functions were evaluated by the Montreal Cognitive Assessment (MoCA), Mini-Mental State Examination (MMSE), and Frontal Assessment Battery (FAB). Apathy and depression were assessed by the Beck Depression Inventory (BDI), Hospital Anxiety and Depression Scale (HADS), and Starskein Apathy Scale. Fatigue was measured using the Fatigue Impact Scale (FIS) and Fatigue Severity Scale (FSS). In addition, patients completed questionnaires to detect sleep disorders: the Ford Insomnia Response to Stress Test (FIRST), Epworth Sleepiness Scale (ESS), Pittsburgh Sleep Quality Index (PSQI), and Insomnia Severity Index (ISI). The EuroQol 5 Dimensions (EQ5D) questionnaire was used to assess quality of life.

The MoCA and MMSE are one of the most commonly used cognitive assessment tools worldwide [18]. The MoCA contains 10 subtests, and the MMSE is composed of 9 subtests. The maximum score in both scales is 30. A score above 26 is considered normal in the MoCA, while a score above 28 is considered normal in the MMSE. Along with these tools, physicians and researchers often use the FAB because it is sensitive to frontal lobe dysfunction and is easy to administer [19]. It consists of 6 subtests, each scored from 0 to 3. A score above 16 is considered normal.

The BDI is a 21-item self-reported questionnaire. Each item is scored on a scale value of 0-3 (maximum total score: 63) [20]. The results are interpreted as follows: 0–9 for no depression, 10–15 for mild depression (minor depression), 16–19 for moderate depression, 20–29 for moderate-to-severe depression, and 30–63 for severe depression [21].

The HADS consists of 2 subscales and detects anxiety and depression. Each subscale includes 7 questions, each scored from 0 to 3. A score of 0–7 indicates no anxiety/depression, a score of 8–10 represents subclinical anxiety/depression, and a score of 11 or above is considered clinically significant anxiety/depression [22].

The Starkstein Apathy Scale includes 14 questions, each scored from 0 to 3. A score of 14 or above indicates clinically significant apathy [23].

The FIS is a 40-item scale that measures the impact of fatigue on patient's quality of life. Each item is scored from 0 to 4 (0: never, 1: once or twice, 2: sometimes, 3: often, 4: every day or almost every day). All statements are divided into 3 subscales: cognitive, physical, and psychosocial (maximum total score of each subscale: 40). An overall score is calculated separately and ranges from 0 to 160. There are no cutoff scores for subscales or the scale as a whole. A higher score indicates greater impact of fatigue on quality of life [24].

The FSS includes 9 statements, each scored on a 7-point scale, from 1 = strongly disagree to 7 = strongly agree. The total score is reported as the mean score of the 9 items. The FSS measures the severity of the patient's fatigue over the past week. The mean score above 4 indicates fatigue [25].

The FIRST includes 9 items asking about the likelihood of sleep disruption due to specific situations. The responses and corresponding scores are as follows: 1 = not likely, 2 = somewhat likely, 3 = moderately likely, and 4 = very likely. The total score ranges from 9 to 36. A higher score indicates a higher likelihood of sleep disruption [26].

The ESS asks patients to rate their likelihood of falling asleep during the day in 8 different situations. Each item is scored on a scale of 0 to 3. The maximum total score is 24. A score above 10 indicates excessive daytime sleepiness [27].

The PSQI is a standardized self-reported questionnaire that assesses sleep quality over the past month. It contains 7 components: sleep duration, disturbances, latency, habitual sleep efficiency, use of sleeping medications, daytime dysfunction due to sleepiness, and overall sleep quality. Each component is scored from 0 to 3, where 0 means no difficulty and 3 means severe difficulty. The maximum total score is 21. A score above 5 indicates sleep disturbances [26].

The ISI is a 7-item self-reported questionnaire that assesses nighttime and daytime components of insomnia. Responses are scored from 0 to 4, where 0 means no problem and 4 means a very severe problem. The maximum total score is 28. The results are interpreted as follows: 0–7 for absence of insomnia, 8–14 for subclinical insomnia, 15–21 for moderate insomnia, and 22–28 for severe insomnia [28].

The EQ5D consists of 6 components: mobility, self-care, usual activities, pain/discomfort, anxiety/depression, and comparison of the current health with

that a year ago. Each component is scored from 1 to 3. A higher score indicates more severe problems. A score of 6 means no problems, a score of 7–12 indicates moderate problems, and a score of 13 or above means severe problems [29].

To collect complete and accurate data on a history of acute COVID-19, we analyzed electronic medical records stored in the QMS database (patients treated in the rehabilitation clinic) and discharge summaries (patients hospitalized in other clinics). We performed neurological examination to assess the neurological status.

During appointments blood samples were taken, and blood serum and plasma were further stored in a biobank.

Statistical analysis was performed using IBM SPSS Statistics 23.0. We used the descriptive statistics, *t* test, Spearman's rank correlation coefficient, linear regression, and odds ratios.

All patients were grouped based on diagnosed disorders. The control group included 26 (28.6%) participants without complaints and objectively diagnosed neurological disorders during the study. Mood disorders were diagnosed in 33 (36.3%) patients. Cognitive disorders during COVID-19 were found in 19 (20.9%) participants. Seven patients had both mood and cognitive disorders. Sleep disorders were observed in 19 (20.9%) participants. During the study, we discovered that 7 patients (7.7%) had the onset of peripheral nervous system diseases after COVID-19; stroke during COVID-19 developed in 3 (3.3%) patients; 3 (3.3%) participants had onset of demyelinating diseases (2 cases of multiple sclerosis and 1 case of acute disseminated encephalomyelitis); onset of the neuromuscular disorder (myasthenia) was reported in 1 (1.1%) patient, and 2 (2.2%) participants presented with persistent anosmia. Patients that experienced onset of neurological disorders during COVID-19 were included only in one group of patients with the corresponding nosology and could not be included in other groups.

This article analyzes data of patients with mood disorders ($n = 33$).

Results

Patient characteristics and features of acute COVID-19 are presented in Tables 1–3.

Table 1. Characteristics of patients from the control and study groups, *n* (%)

Parameter	Control group (*n* = 26)	Patients with mood disorders (*n* = 33)
Sex:		
male	14	10
female	12	23
Mean age, years	60,5 ± 14,1	53,0 ± 14,3
Vaccination:		
no	15	27
before COVID-19	2	4
after COVID-19	9	2
Disease severity:		
mild	5	3
moderate	17	20
severe	4	9
Time after recovery (median), months	7	7
Acute COVID-19 treatment:		
antiviral agents	0 (0%)	2 (6%)
oxygen therapy	19 (73%)	23 (69,7%)
glucocorticoids	18 (69,2%)	23 (69,7%)
Janus kinase inhibitors	3 (11,5%)	11 (33,3%)
monoclonal antibodies	2 (7,6%)	0 (0%)
IL-6 inhibitors	3 (11,5%)	9 (27,3%)
Intensive care unit treatment	1	4

Table 2. Features of the COVID-19 course: symptoms during acute COVID-19, *n* (%)

Symptoms	Control group (*n* = 26)	Patients with mood disorders (*n* = 33)
Fever	21 (80,8%)	32 (97%)
Fatigue	23 (88,4%)	30 (90,9%)
Cough	15 (57,7%)	23 (69,6%)
Dyspnea	19 (73,1%)	21 (63,6%)
Reduced appetite	13 (50%)	22 (66,6%)
Sweating	17 (65,3%)	20 (60,6%)
Chest pain	6 (23,1%)	11 (33,3%)
Rhinitis	5 (19,2%)	7 (21,2%)

Table 3. Features of the COVID-19 course: neurological and somatic symptoms during acute COVID-19 and at the time of examination, *n* (%)

Disorders	Control group (*n* = 26)		Patients with mood disorders (*n* = 33)	
	during COVID-19	at the time of examination	during COVID-19	at the time of examination
Memory impairment (subjective)	8 (30,8%)	7 (26,9%)	16 (48,5%)	15 (45,4%)
Sleep disorder	13 (50%)	6 (23,1%)	24 (72,7%)	18 (54,5%)
Anxiety and depression (subjective)	6 (23,1%)	3 (11,5%)	18 (54,5%)	18 (54,5%)
Headache	10 (38,5%)	5 (19,2%)	15 (45,4%)	5 (15,1%)
Muscle weakness	9 (34,6%)	4 (15,4%)	14 (42,4%)	8 (24,2%)
Back and limb pain	5 (19,2%)	1 (3,8%)	11 (33,3%)	11 (33,3%)
Muscle pain	4 (15,4%)	0 (0%)	11 (33,3%)	3 (9,1%)
Anosmia	13 (50%)	0 (0%)	22 (66,6%)	4 (12,1%)
Ageusia	11 (42,3%)	0 (0%)	19 (57,6%)	3 (9,1%)

Among the participants with mood disorders, depressive disorders and apathy were objectively diagnosed in 12 (36.3%) and 26 (78.7%) patients, respectively. Anxiety was observed in 22 (66.7%) participants, of which 13 (59.1%) and 9 (40.9%) patients had subclinical and clinically significant anxiety, respectively. Fatigue was objectively diagnosed in 20 (60.6%) patients. Signs of mood disorders developed in the study group despite more frequent use of preemptive therapy. This category of patients was prescribed Janus kinase inhibitors 2.9 times more often than the controls (33.3% vs 11.5%, respectively), and IL-6 inhibitors were prescribed 2.4 times more often compared with the control group (27.3% vs 11.5%, respectively). Prescription rates of antiviral agents, oxygen therapy, glucocorticoids, and monoclonal antibodies were almost similar(see percentages in Table 1). We calculated odds ratios for mood disorders development, depending on different symptoms of acute COVID-19. Thus, sleep disorders during the acute phase increased the risk of mood disorders by 2.7 times, the risk of anxiety and depression (subjective) by 2.8 times, the risk of hyposmia or anosmia by 2 times, and the risk of hypogeusia or ageusia by 1.8 times.

Table 4. Results of mood disorders assessment in the examined groups, scores ($M \pm \sigma$)

Screening tool	Control group (n = 26)	Patients with mood disorders (n = 33)	p
BDI	3,885 ± 3,410	10,545 ± 7,268	< 0,001
Starskein Apathy Scale	6,077 ± 4,335	15,909 ± 6,090	< 0,001
HADS (anxiety)	3,962 ± 2,584	8,788 ± 3,959	< 0,001
FIS	36,077 ± 21,779	61,848 ± 29,416	< 0,001
FSS	3,341 ± 1,688	4,278 ± 1,409	0,027

Table 5. Cognitive assessment results in the examined patients, scores ($M \pm \sigma$)

Screening tool	Control group (n = 26)	Patients with mood disorders (n = 26)	Patients with mood and cognitive disorders (n = 7)
MMSE	29,1 ± 1,1	29,5 ± 1,0	27,1 ± 0,9
MoCA	27,6 ± 1,2	28,1 ± 1,3	25,6 ± 2,0
FAB	17,7 ± 0,6	17,6 ± 0,8	17,1 ± 1,2

Table 6. Mean scores for sleep disorders assessment, scores ($M \pm \sigma$)

Screening tool	Control group (n = 26)	Patients with mood disorders (n = 33)	p
FIRST	14,235 ± 3,133	18,167 ± 6,418	0,014
ESS	5,364 ± 3,831	4,962 ± 3,572	–
PSQI	9,118 ± 8,298	14,333 ± 7,883	–
ISI	2,647 ± 2,448	10,625 ± 6,439	< 0,001

It is worth noting that the patients from the study group did not retrospectively report mood disorders before COVID-19. Mean scale scores of the patients from the study and control groups are given in Table 4.

Seven (21.2%) patients were found to have both mood disorders and decreased cognitive functions. Table 5 shows mean cognitive assessment scores in the patients with only mood disorders, those with both cognitive and mood disorders, and the controls. Comparison of cognitive assessment results revealed no significant differences between the patients with mood disorders and the control group.

In addition, the patients were given sleep disorders questionnaires. Among the patients with mood disorders, sleep disorders were observed in 48.5% of cases (16 of 33 patients): of them, 87.5% of the cases (14 patients) were insomnias, and 12.5% of the cases (2 patients) were parasomnias. It should be noted that 56.2% of the patients (9 of 16) retrospectively reported sleep disorders before COVID-19. Our findings are presented in Table 6.

During the data analysis, we found a positive correlation between the BDI, FSS, and FIS scores:

1) BDI and FIS (psychosocial subscale) — high ($r_s = 0.724$; $p < 0.001$);
2) BDI and FIS (cognitive subscale) — moderate ($r_s = 0.544$; $p < 0.001$);
3) BDI and FIS (overall score) — high ($r_s = 0.711$; $p < 0.001$);
4) BDI and FSS — moderate ($r_s = 0.453$; $p = 0.008$).

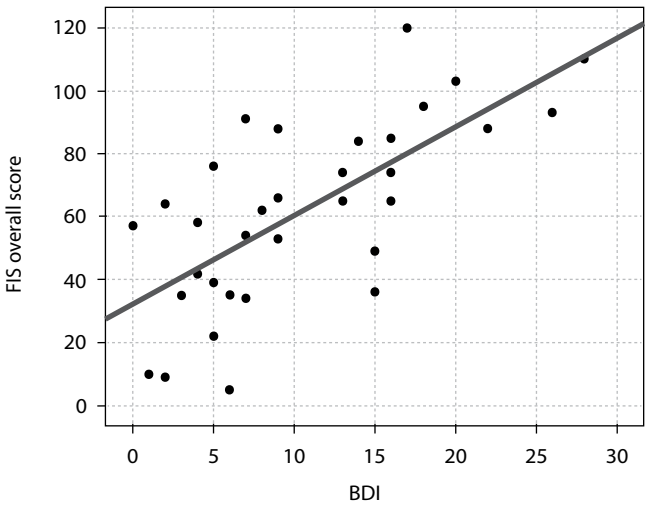

Fig. 1. Correlation of the impact of overall fatigue on quality of life (FIS overall score) and the depression severity (BDI).

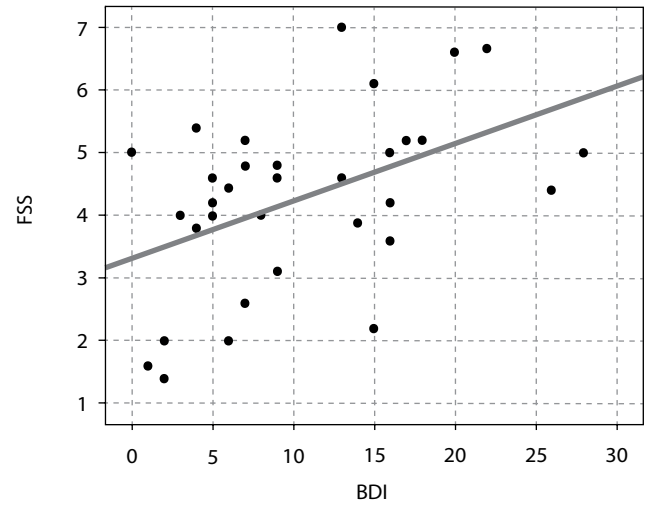

Fig. 2. Correlation of the fatigue severity (FSS score) and the depression severity (BDI score).

We conducted a regression analysis and derived simple linear regression equations for the FIS and FSS scores:
1) Y (FIS overall score) = 2.817 × *x*(BDI) + 32.145;
2) Y (FSS) = 0.091 × *x*(BDI) + 3.324.

Thus, when the BDI score increases by 1, the FIS overall score and the FSS score are expected to increase by 2.817 (Fig. 1) and 0.091 (Fig. 2), respectively, i.e., depressive disorders and fatigue correlate. Patients with higher fatigue levels have more severe depression and vice versa.

The group of patients with anxiety and depression during acute COVID-19 had a significantly higher BDI score at the time of examination (13.3 ± 7.6 and 7.2 ± 5.3; $p = 0.011$).

We found a medium positive correlation between the Starskein Apathy Scale score and the FIS overall score, as well as the FIS psychosocial subscale score ($r_s = 0.350$, $p = 0.046$; $r_s = 0.394$, $p = 0.023$). We conducted a regression analysis and derived a simple linear regression equation:

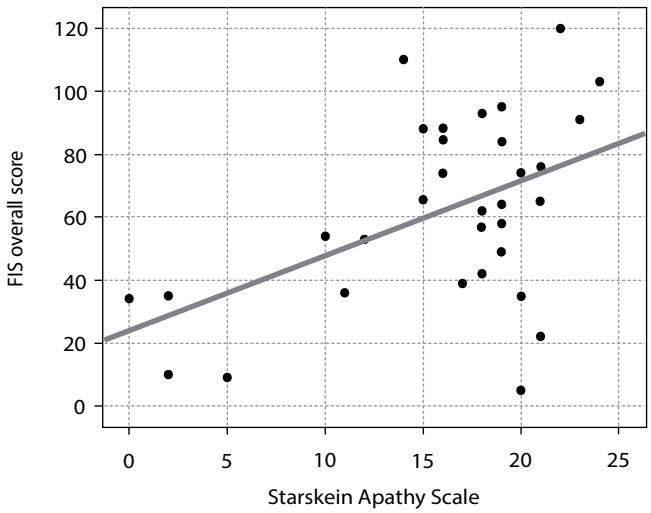

Fig. 3. Correlation of the impact of overall fatigue on quality of life and the apathy severity.

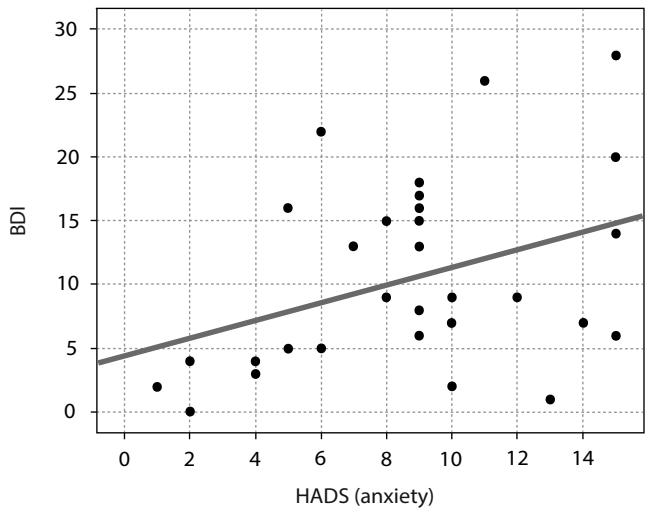

Fig. 4. Correlation of the depression and anxiety severity.

Y (FIS overall score) = 2.356 × *x*(Starskein) + 24.224.

Thus, when the Starskein Apathy Scale score increases by 1, the FIS overall score is expected to increase by 2.365 (Fig. 3), i.e., the impact of fatigue on patients' usual activities increases as apathy becomes more severe, indicating that apathy has a direct negative impact on the fatigue level and quality of life.

We found that based on the HADS (anxiety) and BDI scores, anxiety and depressive disorders have a direct correlation and negatively impact each other ($r_s = 0.366$; $p = 0.036$). Anxiety increased the risk of sleep disorders:

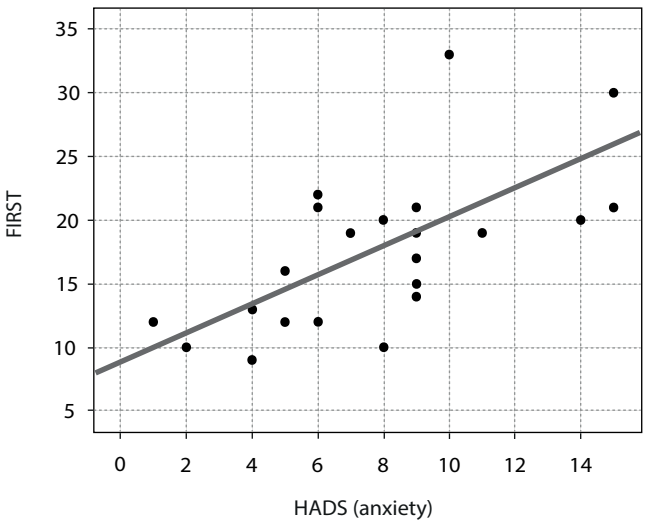

Fig. 5. Relationship between the likelihood of sleep disturbances and anxiety.

there was a significant positive correlation between the HADS (anxiety) and FIRST scores (r_s = 0.683; p = 0.001). However, we found no correlation between depressive disorders and apathy.

After the regression analysis we derived simple linear regression equations for the BDI and FIRST scores:
1. Y (BDI) = 0.686 × x(HADS [anxiety]) + 4.521;
2. Y (FIRST) = 1.143 × x(HADS [anxiety]) + 8.831.

Thus, when the HADS (anxiety) score increases by 1, the BDI score and the FIRST score increase by 0.686 (Fig. 4) and 1.143 (Fig. 5), respectively, i.e., anxiety increases the severity of depressive and sleep disorders, with sleep being affected to a greater extent.

The anxiety severity (based on the HADS [anxiety] score) had no correlation with the impact of fatigue on quality of life (FIS overall score).

In the analyzed group, the severity of depressive disorders, anxiety, and apathy did not depend on sex, age, COVID-19 severity, vaccination, treatment, and cognitive functions. Quality of life decreased both in the patients with mood disorders and the controls (based on the EQ5D score); however, the decline was more significant in the patients with mood disorders (Table 7).

We found a medium positive correlation between the EQ5D and FIS scores (r_s = 0.440; p = 0.01), FSS (r_s = 0.362; p = 0.039). Moreover, there was a medium positive correlation between the EQ5D and BDI scores (r_s = 0.369; p = 0.035). Thus, fatigue and signs of depression negatively impact quality of life in patients with mood disorders.

We found that decline in quality of life did not depend on sex, age, COVID-19 severity, time after recovery, and vaccination in any group.

Discussion

Mood disorders are relatively common PCS manifestations. Huang et al. found that 6 months after recovery, 23% (367 of 1617) of patients reported anxiety or depression [30]. Chen et al. demonstrated that 3 months after recovery, the prevalence of depression and anxiety among 898 patients was 21% and 16.4%, respectively [31]. Our findings show that 36.2% of the patients (33 of 91) developed mood disorders after COVID-19.

Post-COVID-19 fatigue was found to affect up to 65% of patients, with its level correlating with anxiety (HADS score) [10]. Among the examined patients, fatigue was objectively diagnosed in 60.6% of the cases, which is consistent with the literature data. Our correlations, on the other hand, differed slightly: fatigue, depression, and apathy exacerbate symptoms of each other. Nevertheless, we found no statistically significant correlation between fatigue and anxiety.

Table 7. Quality of life assessment in the examined groups, scores (M ± σ)

EQ5D score	Control group (n = 26)	Patients with mood disorders (n = 33)	p
Mobility	1,303 ± 0,467	1,154 ± 0,368	–
Self-care	1,091 ± 0,292	1,038 ± 0,196	–
Usual activities	1,455 ± 0,506	1,154 ± 0,368	0,01
Pain/discomfort	1,697 ± 0,637	1,346 ± 0,485	0,02
Anxiety/depression	1,818 ± 0,584	1,115 ± 0,326	< 0,001
Health comparison	2,545 ± 0,564	2,5 ± 0,51	–
Total score	9,879 ± 1,746	8,308 ± 1,32	< 0,001

Apathy during acute COVID-19 affects up to 92% of patients [32]. This disorder also demonstrates a high prevalence after COVID-19. Calabria et al. assessed apathy after COVID-19 and compared their findings to retrospective self-reported data. The study included 136 COVID-19 patients. The number of patients reporting apathy increased from 23 (16.9%) before COVID-19 to 85 (62.5%) after COVID-19 [33]. Our findings revealed that apathy was reported in 28.6% of the cases (26 of 91 patients) and was the most common mood disorder. The study group included patients without preexisting disorders according to the retrospective self-reported data.

Neuroinflammation and increased cytokine levels are said to be key mechanisms underlying mood disorders during COVID-19 [16, 17]. However, we discovered that these disorders developed in the study group despite more frequent use of preemptive therapy, including IL-6 inhibitors. Cytokine levels might have decreased due to conservative therapy but did not return to the normal range, thus, leading to symptoms of depression, anxiety, and apathy. It is important to note that such medications as Janus kinase inhibitors, IL-6 inhibitors, and monoclonal antibodies, are often prescribed in case of severe deterioration of the general condition that definitely affect person's mood. To make our findings objective, we plan to measure blood serum levels of IL-6 at the first appointment and at 6 months.

The severity of mood disorders is expected to gradually decrease over time. Huang et al. investigated the prevalence of anxiety and depression among 511 patients at 6 and 12 months after COVID-19. The prevalence of anxiety decreased from 13.31% (at 6 months) to 6.26% (at 12 months), whereas the prevalence of depression decreased from 20.35% to 11.94% [34]. We observed a slightly higher prevalence of anxiety 7 months after COVID-19 and a lower prevalence of depressive disorders: 24.1% (22 of 91 patients) and 13.1% (12 of 91 patients), respectively. To assess changes in mood disorders over time, the repeated appointment is scheduled 6 months after the first appointment.

Ortelli et al. showed a direct correlation between apathy and depression [16]; however, we found no such correlation.

Conclusion

Of 91 examined patients, 65 (71.4%) had PCS. Mood disorders occurred in 33 (50.8%) patients, with apathy (26 patients, 78.7%) and anxiety (22 patients, 66.7%) being the most common. Depression was less common: 12 (36.3%) cases.

Risk factors for mood disorders as PCS manifestations include sleep disorders, anxiety and depression, hyposmia and anosmia, hypogeusia and ageusia during acute COVID-19. The fatigue level correlates with the seve rity of depressive disorders and apathy. In the patients from the study group, the anxiety severity had a direct correlation with the depression severity. Patients with anxiety had an increased risk of sleep disorders. The severity of depressive disorders and fatigue negatively impacted patients' quality of life. We should also note that the patients did not report any mood disorders before COVID-19.

Due to the high prevalence of mood disorders among PCS manifestations and their impact on quality of life, these disorders should be diagnosed and treated early on, involving psychiatrists and sleep physicians if needed.

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MRI-Guided Focused Ultrasound in Cervical Dystonia

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Abstract

Introduction. *MRI-guided focused ultrasound (MRgFUS) is approved for management of various movement disorders, primarily essential tremor and Parkinson's disease (PD), with favorable long-term outcomes in numerous patients worldwide. However, few case studies describe the use of this modality for symptomatic treatment of dystonias that, as the third most common movement disorder, may be rather disabling.*
Objective: *To improve outcomes in patients with cervical dystonia (CD) using MRgFUS.*
Materials and methods. *We retrospectively analyzed 13 cases of various CD types managed with MRgFUS in single or multiple sessions. The mean age of the patients was 42 [39; 53] years. The Toronto Western Spasmodic Torticollis Rating Scale (TWSTRS) was used to assess patients' statuses and severity of CD symptoms during therapy and the last available observation period. The targets included the pallidothalamic tract and the thalamic ventral oralis complex nucleus or their combination.*
Results. *The mean follow-up period was 13.3 ± 3.4 months (July 2021 to April 2023). The mean CD severity sum score (TWSTRS score) was 22 [16; 25] before MRgFUS and 6 [4; 9] in the last observation. Therefore, we report 70.6% [55.6; 76.5] improvement (paired samples t-test p = 0.0025).*
Conclusion. *Available data evidence that MRgFUS is efficient and sufficiently safe for symptomatic treatment in pharmacoresistant CD patients. A number of vital aspects of MRgFUS have to be specified in larger CD cohorts in the long-term follow-up.*

Keywords: *MRI-guided focused ultrasound; cervical dystonia; thalamic ventral oralis complex nucleus; pallidothalamic tract; ventral interposed nucleus; pallidothalamic tractotomy*

Ethics approval. The study was conducted with the informed consent of the patients. The research protocol was approved by the local Ethics Committee of the Research Center of Neurology (protocol No. 1-8/23, January 25, 2023).

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Conflict of interest. The authors declare no apparent or potential conflicts of interest related to the publication of this article.

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Фокусированный ультразвук под контролем МРТ в лечении цервикальной дистонии

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Аннотация

Введение. *Фокусированный ультразвук под контролем МРТ (МР-ФУЗ) одобрен для лечения различных расстройств движений, в первую очередь эссенциального тремора и болезни Паркинсона, причём такие вмешательства, выполненные в мире на многих сотнях пациентов, сопровождаются благоприятными долгосрочными результатами. Однако в доступной литературе описаны лишь единичные случаи использования данной технологии для коррекции симптомов дистоний, которые могут быть весьма инвалидизирующими и по распространённости занимают третье место среди всех клинических форм расстройств движений.*

Цель *исследования — улучшение результатов лечения пациентов с цервикальной дистонией (ЦД) при помощи технологии МР-ФУЗ.*
Материалы и методы. *Ретроспективно проанализированы данные 13 пациентов с различными типами ЦД, которым проводилось лечение с помощью МР-ФУЗ поэтапно или одномоментно. Средний возраст пациентов составил 42 [39; 53] года. Состояние пациентов и выраженность симптомов ЦД оценивали по шкале спастической кривошеи Западного Торонто (TWSTRS, оценка тяжести ЦД) во время лечения и в последний доступный период наблюдения. В качестве мишеней использовали паллидоталамический тракт и вентрооральное ядро таламуса или их комбинацию.*
Результаты. *Средний период клинического наблюдения за пациентами составил 13,3 ± 3,4 мес (с июля 2021 г. по апрель 2023 г.). Средняя сумма баллов по шкале TWSTRS (оценка тяжести ЦД) составила 22 [16; 25] до МР-ФУЗ и 6 [4; 9] — в последний доступный период наблюдения. Таким образом, достигнуто улучшение на 70,6% [55,6; 76,5] (парный критерий Вилкоксона p = 0,0025).*
Заключение. *Имеющиеся данные позволяют говорить, что МР-ФУЗ является эффективным и достаточно безопасным методом коррекции симптомов ЦД, резистентной к фармакологическим методам лечения. Многие важные аспекты применения МР-ФУЗ у пациентов с ЦД ещё предстоит уточнить на более обширных когортах больных в рамках многолетнего катамнестического наблюдения.*

Ключевые слова: *фокусированный ультразвук под контролем магнитно-резонансной томографии; цервикальная дистония; вентрооральное ядро таламуса; паллидоталамический тракт; вентральное промежуточное ядро таламуса; паллидоталамическая тракотомия*

Этическое утверждение. Исследование проводилось при добровольном информированном согласии пациентов. Протокол исследования одобрен локальным этическим комитетом ФГБНУ НЦН (протокол № 1-8/23 от 25.01.2023).

Источник финансирования. Авторы заявляют об отсутствии внешних источников финансирования при проведении исследования.

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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Introduction

Cervical dystonia (CD) is the most prevalent (≤ 50%) clinical dystonia type. CD is a focal dystonia, with involuntary tonic contractions or intermittent spasms of neck muscles and resulting abnormal neck and head position and/or head tremor [1–5]. The CD prevalence is 1.2–5.7 per 1,000,000 person-years [2], while the CD incidence is 8–12 per 1,000,000 person-years [3], with the manifestation peak falling at the age of 30–50 years [2–4]. The disorder is twice as common in female patients [2]. CD etiology varies. There are congenital (idiopathic) and acquired dystonias [5–7]. Idiopathic CD is shown to be related with *DYT2*, *DYT13*, *DYT23*, *DYT24*, *DYT25*, and other loci mutations [8]. Acquired dystonias develop in patients with the brainstem and basal ganglia lesions of various origin, as a result of long-term use of dopamine receptor antagonists, etc. [4, 8]. Patients with dystonia manifestations often demonstrate functional (psychogenic) disease, which requires special attention and diagnosis experience [9].

CD symptoms typically progress within the first several years up to plateau [4, 5]. Clinically, CD implies the abnormal position of the head (torticollis, torticaput, laterocollis, laterocaput, anterocollis, anterocaput, retrocollis, retrocaput), the neck, and the shoulders with dystonic head tremor aggravated in voluntary movement, fatigue, and emotional strain. Many patients use sensory tricks, such as touching the chin or cheek, to reduce symptoms.. CD is often complicated with depression, anxiety, and phobias and makes patients highly incapable, and limits their daily living and social life [3–5, 10]. While several scales are used to assess CD symptom severity, the Toronto Western Spasmodic Torticollis Rating Scale (TWSTRS) is the fittest and most widely used [11].

Recently, CD management has transformed dramatically, from exercise therapy, pharmaceutical treatment, and local muscle surgeries through stereotactic ablation and deep brain stimulation (DBS) to innovative non-invasive approaches like MRgFUS [6, 7, 12–17]. Oral agents (clonazepam, anticholinergic agents, myorelaxant agents, etc.) are as a rule ineffective and have significant side effects in therapeutically necessary higher doses [4, 16]. Currently, the gold standard of CD therapy is the use of botulinum toxin type A to treat abnormal movements and to relieve pain [6, 7]. Its disadvantages include the need for repetitive injections every 3–4 months, inadequate effectiveness that depends on CD signs and symptoms, effectiveness decrease, and resistance in some patients [16, 18].

CD surgical treatment dates back to 1641 when the German surgeon named Isaac Minnius sectioned the sternocleidomastoid muscle [10]. Such local interven-

tions had been popular before mid-20th century when the rise of functional stereotactic surgery revolutionized CD neurosurgical treatment and laid the foundation of today practice including DBS and MRgFUS. Soviet and Russian neurosurgeons gained abundant experience in destructive surgery for dystonia [10, 19–21].

In the 1970s, based on W. Hess's experimental results, R. Hassler and G. Dieckmann attempted to consider CD clinical signs and symptoms and to select destruction targets in order to involve the pallidothalamic tract (PTT) in H1 Forel's field (in torticollis) and the thalamic ventral oralis (Vo) complex nucleus (in laterocollis) contralaterally to the head turn side [22, 23]. Following up 112 CD patients post ventro-lateral thalamotomy, E.I. Kandel concluded that the bilateral intervention was more efficient, especially in patients with head hyperkynesias [19]. Follow-up results correspond to the current understanding of CD pathogenesis [14, 24, 25].

Prior to DBS large-scale implementation, destructive surgery was the leading approach in CD symptomatic treatment with effectiveness of 50–70%. Such interventions were performed mostly unilaterally because bilateral destruction was typically (20–70%) complicated by dysarthria, dysphagia, ataxia, and symptomatic parkinsonism [12, 14, 24].

In the late 20th century, DBS became the leading approach in CD neurosurgical treatment [15, 26, 27]. Right and left globus pallidus internus stimulation (GPI-DBS) is the modality commonly used in patients with CD who did not respond to pharmaceutical and botulinum toxin therapies. Improvement after bilateral GPI-DBS may vary from TWSTRS score 27.8% [15] to TWSTRS score 51.4% [27] or 66.6% [28], depending on stimulation parameters, patient cohorts, and observation periods. According to J. Volkman et al., 10% of the patients did not respond to GPI-DBS despite multiply varied stimulation parameters [17].

The clinical implementation of MRgFUS to treat movement disorders revitalized functional brain destruction. Recently, we have accumulated extensive evidence of MRgFUS safety and efficiency in patients with essential tremor and Parkinson's disease (PD) [29–32]. However, by the moment, we have found only single reports on MRgFUS to treat dystonia [13, 33, 34]. We are presenting our own experience of MRgFUS use to manage patients with CD.

Objective. To improve outcomes in patients with CD using MRgFUS.

Materials and methods

We retrospectively analyzed 13 cases of various CD types managed with MRgFUS in single or multiple sessions. The mean age of the patients was 42 (39; 53) years (Fig. 1). All of them had no family history of dystonia. The disorder manifested as dystonic hand tremor at early age with subsequent cervical involvement in 2 patients. In other cases, hyperkynesia manifested as isolated CD and was not combined with any other movement disorders.

All the CD patients were refractory to botulinum toxin therapy after several courses. DBS was rejected due to patients' disregard of head mechanical implants (patients' refusal) or lack of access to the medical centers that could adjust stimulation parameters.

TWSTRS was used to assess patients' statuses and severity of CD symptoms during therapy and the last available observation period. The Hospital Anxiety and Depression Scale (HADS) and the Montgomery–Åsberg Depression Rating Scale (MADRS) were used to assess anxiety and depression [11].

MRgFUS CD symptomatic treatment was conducted at V.S. Buzaev Memorial International Medical Center. We used ExAblate Model 4000 (Insightec v.7.0.404, Insightec), with 1024 ultrasound generators, and GE Optima MRI scanner (MR450W, 1.5 T). The standard procedure of preparation for MRgFUS was performed in all the patients.

Targets included PTT and/or Vo (see Table). As there is no unified standard or rationale, targets are selected based on published experience in specific CD cases. Reverse ultrasound exposures allow to model effects in a particular brain area and to select the most efficient target for the patient. The targets were sonicated at least twice at temperature over 55ºC. The median MRgFUS time was 117 (79; 139) min; the median sonication number was 12 (11; 14.5). The energy range was 20,096–35,731 J in a temperature range 54–62ºC.

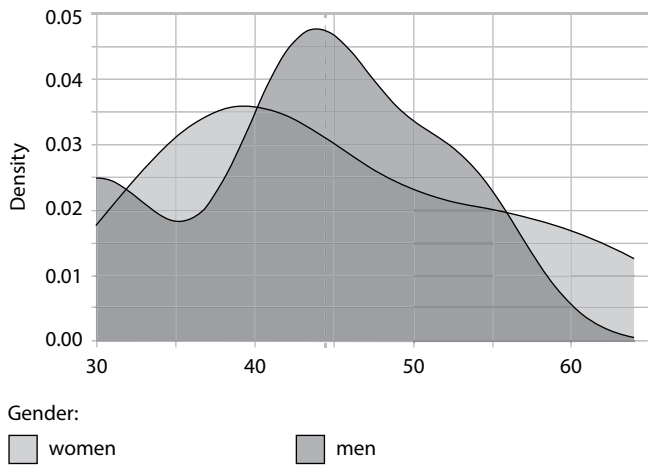

Fig. 1. Age distribution of operated CD patients.

Characteristics of included CD patients

CD clinical signs and symptoms	МР-ФУЗ MRgFUS age	CD onset age	Sex	TWSTRS score — assessed CD severity		Target	
				pretreatment	post treatment	right hemisphere	left hemisphere
Right torticollis, right torticollis, head tremor	42	4	M	16	9		PTT VO
Left torticollis, left laterocollis, head tremor	53	33	M	22	6	PTT VO	
Right torticollis, head tremor	53	43	F	22	4		PTT VO
Right torticollis, head tremor	36	31	F	23	6		PTT VO
Right torticollis, head tremor	39	23	F	4	0		PTT VO
Left torticollis, right laterocollis, orofacial dystonia	39	37	M	27	9	VO	PTT
Left torticollis, left laterocollis, head tremor	42	18	F	17	4	PTT VO	
Right torticollis, left laterocollis, orofacial dystonia	57	26	M	29	15	VO	PTT
Left torticollis, retrocollis	46	33	F	26	10	PTT	VO
Left torticollis, head tremor	30	15	M	9	4	PTT	
Right torticollis, head tremor	57	27	F	13	2		PTT VO
Right laterocollis, head tremor	32	17	F	17	5	VO	PTT
Right laterocollis	47	27	F	25	14		PTT

Note. M, male; F, female.

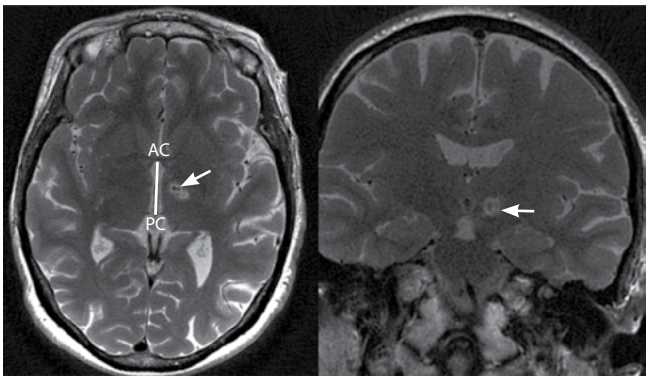

Fig. 2. Axial and coronal MR images in 30 days after right PTT MRgFUS destruction.
The arrow indicates the destruction focus.

The MRgFUS procedure included control brain MRI scan (T2-weighted; axial, sagittal, and coronal views; 2-mm thick slices). Intraoperative imaging showed no signs of hemorrhage or non-target heat in any patient. Follow-up brain MRI scans in 2 h and 24 h, and 30 days revealed slight marginal edema (1–3 mm) and necrotic foci at the sites of sonication (Fig. 2).

Results

The mean follow-up period was 13.3 ± 3.4 months (July 2021 to April 2023). The mean CD severity assessment (TWSTRS score) was 22 [16; 25] before MRgFUS and 6 [4; 9] in the last available observation. Therefore, we found 70.6% [55.6; 76.5] improvement (paired samples t-test $p = 0.0025$).

Six patients demonstrated mild side effects including gait disorders and postural unsteadiness for 3 weeks. Two patients had significant logorrhea totally reversed on quetiapine (25 mg/day) within a month. Two female patients noted memory deterioration in 1 month post MRgFUS followed by gradual recovery by the end of the follow-up year 1. Two patients showed altered handwriting with slight micrography and gradual postoperative recovery.

One patient with significant head tremor showed recurrent hyperkynesia in 6 months post MRgFUS. This patient received re-intervention in 9 months post initial procedure, with no recurrent head tremor during next 4 months.

The patients reported evident positive changes in their daily living and their social and professional lives throughout the follow-up period. Three patients got better-paid job positions; one patient changed nighttime IT position for academic lecturing; one female patient was proposed to get married; one patient stopped having the status of disabled person; one patient resumed working as an operating surgeon; two patients resumed their occupations.

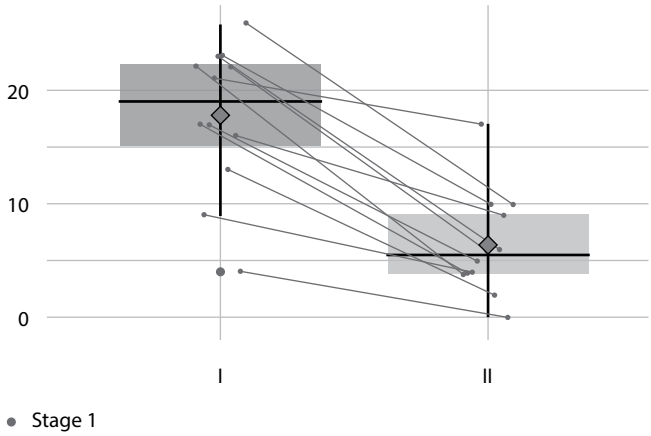

Fig. 3. TWSTRS scores pre (I) and immediately post (II) MRgFUS intervention.

Discussion

Management of CD patients remains a challenge for neurologists [4, 5, 16, 18]. DBS is the best option that can significantly relieve CD symptoms [15, 26], but it is not widely used due to its complexity and low accessibility. MRgFUS can become an alternative, innovative, and non-invasive method of functional neurosurgery in this population though the experience of its use for CD (unlike PD and essential tremor) is especially limited and, according to the guidelines, MRgFUS is no method of choice [13, 33, 34].

In 2021, S. Horisawa et al. published an open-label pilot study and showed that Vo MRgFUS significantly relieved focal hand dystonia in 10 patients, with mild dysarthria in 1 patient as the only adverse event in 12 months [13]. R. Jamora et al. demonstrated general improvement in 3 patients with X-linked dystonia-parkinsonism (XDP) post MRgFUS Vo-thalamotomy [34]. The XDP-MDSP scores improved by 36.2% in 6 months and by 30.1% in 12 months. However, central pain syndrome manifested in 2 patients in 2–7 months post treatment.

We have described our own, first experience in Russia of the MRgFUS use for CD (in 13 patients with follow-up period over 1 year and TWSTRS improvement by 70.6%). Due to recurrent tremor, one patient received re-intervention in 9 months after initial procedure. Complications were relatively mild and resolved by the end of follow-up year 1. We selected PTT and/or Vo as targets, basing on published intervention outcomes in relevant patient categories [13, 14, 33, 34]. So, PTT destruction relieves CD symptoms due to disruption of cortico-basal and thalamo-cortical pathways and modulation of thalamic efferent stimuli while Vo interventions may be useful in patients with laterocollis [25, 35].

Available data allow us to consider MRgFUS as an efficient method to treat symptoms of pharmacoresistant CD. A number of vital aspects of MRgFUS have to be specified in larger CD cohorts in long-term follow-up. This method may be considered as advanced in management of patients with other dystonias, which has to be proven in special studies including multicenter trials. We may expect that, introduced into clinical practice more widely, this mini-invasive method will be gradually used in patients with movement disorders more extensively.

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Long-Term Outcomes of Management of Inferior Alveolar Neuropathy Following Orthognatic Surgeries in Patients with Mandibular Anomalies and Deformities

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Abstract

Introduction. *Orthognatic surgery is a routine method to manage mandibular anomalies and deformities.*
Objective: *To assess long-term outcomes of rhythmic peripheral magnetic stimulation (rPMS) in patients with neuropathy of the inferior alveolar nerve (IAN) resulting from the surgical treatment of mandibular anomalies and deformities.*
Materials and methods. *The study included 8 males and 16 females aged 32 ± 12 years with IAN neuropathy following the surgical treatment of mandibular anomalies and deformities. Therapeutic rPMS was performed with the Neuro-MS magnetic stimulator (Neurosoft, Ivanovo, Ivanovo Region, Russian Federation). Trigeminal and brainstem acoustic evoked potentials (EPs) were registered with Neuro-MVP (Neurosoft) to assess rPMS both at baseline (in 10 days) and in long term (in 18 ± 2 months).*
Results. *Sensory disorders and pain prevailed in postoperative IAN neuropathy. Sensory disorders improved in 20 patients following 10-day rPMS. The clinical effect persisted in re-assessment. In long term, acoustic brainstem EPs normalized and trigeminal EPs did not change negatively.*
Conclusion. *The use of rPMS in IAN neuropathy following orthognatic surgeries contributes to the functional improvement and stabilization of the peripheral and central brainstem and the trigeminal system.*

Keywords: *IAN neuropathy; brainstem auditory evoked potentials; trigeminal evoked potentials; orthognatic surgery*

Ethics approval. The study was conducted after receiving the informed consent of the patients. The study protocol was approved by the Ethics Committee of the Research Center of Neurology (Protocol No. 11/4-19, 20 November 2019).

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Отдалённые результаты лечения нейропатии нижних луночковых нервов после ортогнатической коррекции аномалий и деформаций нижней челюсти

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Аннотация

Введение. *Ортогнатические операции являются наиболее распространённым методом лечения аномалий и деформаций лицевого черепа.*
Цель *исследования — оценка отдалённых результатов применения ритмической периферической магнитной стимуляции (рПМС) при нейропатии нижних луночковых нервов (НЛН), возникшей в результате хирургического лечения аномалий и деформаций нижней челюсти.*
Материалы и методы. *В исследование были включены 8 мужчин и 16 женщин в возрасте 32 ± 12 лет с нейропатией НЛН после ортогнатической коррекции аномалий и деформаций нижней челюсти. Для лечебной рПМС использовали магнитный стимулятор «Нейро-МС» («Нейрософт»). При оценке отдалённых результатов эффективности рПМС (через 18 ± 2 мес), как и при первом исследовании (через 10 дней), регистрировали акустические стволовые и тригеминальные вызванные потенциалы на приборе «Нейро-МВП» («Нейрософт»).*
Результаты. *В неврологической картине постоперационной нейропатии НЛН преобладали чувствительные и болевые нарушения. Клинический эффект в виде уменьшения чувствительных нарушений после 10-дневного курса рПМС наблюдался у 20 пациентов и сохранялся при повторном обследовании. В отдалённом периоде также отмечены нормализация параметров акустических стволовых вызванных потенциалов и отсутствие отрицательных изменений при исследовании тригеминальных вызванных потенциалов.*
Заключение. *Применение рПМС при нейропатии НЛН, возникшей после ортогнатических операций, способствует улучшению и стабилизации функции периферических и центральных структур ствола мозга и тригеминальной системы.*

Ключевые слова: *нейропатия нижних луночковых нервов; акустические стволовые вызванные потенциалы; тригеминальные вызванные потенциалы; ортогнатические операции*

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Introduction

Orthognatic surgery is a routine method to manage mandibular deformities. Its benefits include easier mastication, reduced tenderness in the temporomandibular joints, and better facial esthetics. Necessary osteotomy is performed in close proximity from the inferior alveolar nerve (IAN) [1]. Sensory disorders (numbness or pain in the lower lip, the chin, the teeth, and the gums) are reported in 16.2% of the patients. Though usually temporary, paresthesiae may be permanent [2].

Following orthognatic surgeries for mandibular anomalies and deformities, prevalence of IAN neuropathy varies from 1.3% to 18%. Postoperative sensory disorders in the lower lip and the chin develop in 9–85% of the patients [1, 3, 4].

IAN is often injured as a result of interventions in the mandible and the facial soft tissues or IAN injury [4, 5]. IAN injury may imply full or partial nerve dissection, strain, compression, crush, or ischemia [6]. Depending on the severity of nerve fiber injury, neuropraxia, axonotmesis, or neurotmesis may develop [7]. Damage

to the myelin sheath causes demyelination that impairs signal conduction and thus sensitivity. Demyelination of varied severity develops in neuropraxia and axonotmesis [7–9].

Specific IAN injury symptoms include loss of sensitivity in the lower lip on the affected side, the chin, and the gum. When occluding their teeth, the patient feels wrenching pain and discomfort, which affects their quality of life, mastication, speech, and facial expressions while the patient is often unsatisfied with management [1, 2, 10, 11].

Management of traumatic trigeminal neuropathy is a challenge. Physiotherapy is recommended in combination with antidepressants or (rarely) as a single approach. Use of rhythmic transcranial magnetic stimulation (rTMS) is limited with variability of protocols and outcomes [12, 13]. Rhythmic peripheral magnetic stimulation (rPMS) can modulate cortical chain reactions and cortical spinal excitability. Unlike rTMS, rPMS exposes certain body parts rather than their projections on the brain cortex.

Unlike electric stimulation, magnetic stimulation exposes deeper tissues, accelerating neurotransmission and not activating any skin receptors [14, 15].

rPMS is typically used to manage pain and spasticity. However, the published studies covered only a few cases and various protocols [16–18].

Noteworthily, there are no unequivocal recommendations on the use of magnetic stimulation in patients with traumatic trigeminal neuropathy yet [19]. Some studies show that magnetic stimulation relieves pain and accelerates regeneration of the injured nerves [20, 21].

Objective: to assess long-term rPMS outcomes in patients with IAN neuropathy resulting from the surgical treatment of mandibular anomalies and deformities.

Materials and methods

The study included 8 males and 16 females aged 32 ± 12 years with IAN neuropathy following the surgical treatment of mandibular anomalies and deformities [10]. Approved by the Ethics Committee of the Research Center of Neurology (Protocol No. 11/4-19, 11/20/2019), the study continues those published before [10, 22].

Seventeen patients had permanent, similarly intense, aching or contracting pain. The pain was localized individually in the same area: the lower lip, the chin, a mandibular tooth or several mandibular teeth, an alveolar mandibular site. The pain irradiated subzygomatically (posteriorly) in all the patients. Four patients did not feel the painful side of the lower lip they consequently bit when eating or speaking. Besides, the patients complained of gum contracting feeling. Those patients felt hypersthesia with pain, cold, and tactile hyperpathia and warmth hypesthesia in the chin, the lower lip, and the mandibular gum and teeth. In palpation of the pain area, they also felt sharp tenderness. Three patients with IAN neuropathy felt stabbing, burning pain in the lower lip. All the patients reported decreased sensitivity in the IAN innervated area.

Therapeutic rPMS was performed with the Neuro-MS magnetic stimulator (Neurosoft, Ivanovo, Ivanovo Region, Russian Federation). The procedures were performed daily for 15–20 min during 10 days, with 1.0–1.5 T stimuli and 1 Hz frequency. The patients received no pharmaceuticals to stimulate reparation and to improve neurological functions [10].

The patients were followed up in 18±2 months to re-assess long-term rPMS efficiency. Evoked potentials (EPs) including brainstem auditory evoked potentials (BAEPs) and trigeminal EPs (TEPs) were recorded by the Neuro-MEP (Neurosoft, Ivanovo, Ivanovo Region, Russian Federation) [10, 22, 23].

Results

Immediately post 10-day rPMS treatment, 20 patients demonstrated significantly decreased sensory disorders while 4 patients still showed facial paresthesiae. BAEPs changed positively, but TEPs did not change significantly [10, 22].

Table 1. BAEPs before treatment and in 18 ± 2 months after rPMS (median)

Group	Latency, msec			Interpeak interval, msec			Amplitude, μV		
	I	III	V	I–III	III–V	I–V	I	III	V
Normal	1,7 ± 0,1	3,9 ± 0,2	5,7 ± 0,2	2,1 ± 0,2	1,9 ± 0,2	4,0 ± 0,2	0,3 ± 0,1	0,2 ± 0,1	0,4 ± 0,2
Right and left ears:									
post 10-day rPMS treatment	1,6	3,5	5,4	2,0	1,9	3,9	0,3	0,3	0,5
in 18 months post rPMS	1,6	3,6	5,5	2,0	1,9	3,9	0,3	0,3	0,5

Note. I, III, V, BAEP peaks.

Table 2. TEP parameters before and in 18 ± 2 months after rPMS treatment (median)

Group	Threshold, mA	TEP components, msec			Amplitude, μV	
		P1	N1	P2	P1-N1, μV	N1-P2, μV
Normal	5,7	19,2	33,0	49,0	1,9	1,9
Left and right stimulation:						
post 10-day rPMS treatment	5,1	19,8	31,3	43,5	2,0	1,9
in 18 months post rPMS treatment	5,2	20,1	31,7	44,5	2,0	1,9

Clinically, we noted improvement, with reversed sensitivity disorders and better subjective status in 83% of the patients in 18 ± 2 months. BAEPs normalized as well (Table 1), which may indicate rPMS stabilizing general processes and auditory brainstem response.

Post initial 10-day rPMS treatment, TEP changes indicated non-significant bilateral trigeminal dysfunction (Table 2). In 18 ± 2 months, TEPs did not show any negative changes. Therefore, rPMS use contributes to the improvement and the stabilization of the trigeminal system and the brainstem in IAN neuropathy.

Discussion

rPMS is a method of noninvasive stimulation of nerves, muscles, spinal nerve roots, and the autonomic nervous system. rPMS affects excitability of sensory terminals under the coil, which causes functional neuron changes and neuroplasticity. rPMS is relatively painless and may be easily used in clinical setting. Currently, rPMS is widely used for rehabilitation [24].

Magnetic stimulation acts due to the generated magnetic field that induces the electric field, which depolarizes axons. However, the mechanisms of magnetic fields acting to peripheral nerves are still unclear [25, 26]. The effect of the magnetic field on cells may be explained by its impact on the molecular structure of excitable cell membranes followed by the change in the function of insert ion specific channels [27]. Voltage-controlled potassium, sodium, and calcium ion channels are affected by the magnetic field, which makes neurons highly sensitive to the effects of the magnetic field [28–30].

Another possible mechanism is the magnetocaloric effect resulting from magnetic nanoparticle activity in the external magnetic field [31]. Despite lack of evidence for the relation between the magnetocaloric effect and nerve regeneration in the magnetic field, the experimental studies showed that temperature elevation to 37–42°C may positively affect neuron number increase [32].

The effect of the magnetic field on peripheral nerves is also associated with increase in growth factors and decrease in pro-inflammatory factors. rPMS has vasoprotective, anti-inflammatory, and antiedematic effects and improves trophicity in the injured site. rPMS is getting wider renowned as a method of neuromodulation in sensomotor disorders.

Our earlier study demonstrated efficiency of rPMS in patients with traumatic trigeminal neuropathy. Restored sensitivity as a positive effect of 10-day rPMS treatment significantly improved quality of life in most patients [10, 22].

The positive rPMS outcomes in patients with IAN neuropathy after orthognatic surgeries for mandibular anomalies and deformities in long term (18 ± 2 months) substantiate necessity of this method as part of individual rehabilitation.

Nevertheless, the development of main principles of rPMS use post orthognatic surgeries requires methodological and clinical studies including larger samples.

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Long-term Intracerebroventricular Administration of Ouabain Causes Motor Impairments in C57Bl/6 Mice

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Abstract

Introduction. *Cardiac glycosides are natural ligands of Na^+/K^+-ATPase, which regulate its activity and signaling. Intracerebroventricular administration of ouabain has been previously shown to induce hyperlocomotion in C57Bl/6 mice via a decrease in the rate of dopamine reuptake from the synaptic cleft.*
Materials and methods. *This study involved forty C57BL/6 mice. 1.5 μL of 50 μM ouabain was administered daily into the left lateral cerebral ventricle over the course of 4 days. On day 5, open field, beam balance, and ladder rung walking tests were performed to assess the locomotor activity and motor impairments in the mice. We evaluated changes in the activation of signaling cascades, ratios of proapoptotic and antiapoptotic proteins, and the amount of α1 and α3 isoforms of the Na^+/K^+-ATPase α-subunit in brain tissue using Western blotting. Na^+/K^+-ATPase activity was evaluated in the crude synaptosomal fractions of the brain tissues.*
Results. *We observed hyperlocomotion and stereotypic behavior during the open field test 24 hours after the last injection of ouabain. On day 5, the completion time and the number of errors made in the beam balance and ladder rung walking tests increased in the mice that received ouabain. Akt kinase activity decreased in the striatum, whereas the ratio of proapoptotic and antiapoptotic proteins and the number of Na^+/K^+-ATPase α-subunits did not change. Na^+/K^+-ATPase activity increased in the striatum and decreased in the brainstem.*
Conclusions. *Long-term exposure to ouabain causes motor impairments mediated by changes in the activation of signaling cascades in dopaminergic neurons.*

Keywords: *Na^+/K^+-ATPase; ouabain; cardiac glycosides; dopaminergic system*

Ethics approval. Authors confirm compliance with institutional and national standards for the use of laboratory animals in accordance with «Consensus Author Guidelines for Animal Use» (IAVES, 23 July 2010). The research protocol was approved by the Ethics Committee of the St. Petersburg State University (protocol No. 131-03-1, March 25, 2019).

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Conflict of interest. The authors declare no apparent or potential conflicts of interest related to the publication of this article.

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Хроническое внутрижелудочковое введение уабаина вызывает моторные нарушения у мышей линии C57Bl/6

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Аннотация

Введение. *Кардиотонические стероиды являются природными лигандами Na+,K+-АТФазы, которые регулируют её активность и сигнальную функцию. Ранее было показано, что уабаин при однократном внутрижелудочковом введении вызывает гиперлокомоцию у мышей линии C57Bl/6 вследствие уменьшения скорости обратного захвата дофамина из синаптической щели.*

Материалы и методы. *В исследовании были использованы 40 мышей линии C57Bl/6. На протяжении 4 дней животным ежедневно вводили 1,5 мкл 50 мкМ уабаина в латеральный желудочек головного мозга. На 5-й день производили оценку локомоторной активности и моторных нарушений при помощи тестов «открытое поле», «удержание на планке» и «лесенка с перекладинами». В тканях мозга оценивали изменение активации сигнальных каскадов, соотношения про- и антиапоптотических белков, а также количества α1- и α3-изоформ α-субъединицы Na^+,K^+-АТФазы при помощи иммуноблоттинга. Активность Na^+,K^+-АТФазы оценивали в грубой синаптосомальной фракции тканей мозга.*

Результаты. *Через 24 ч после последнего введения уабаина у мышей наблюдались гиперлокомоция и стереотипность движений в тесте «открытое поле». У мышей, получавших уабаин, на 5-й день эксперимента увеличивалось время прохождения и количество ошибок в тестах «лесенка с перекладинами» и «удержание на планке». В стриатуме мышей активность киназы Akt снижалась, соотношение про- и антиапоптотических белков не менялось, как и количество α-субъединиц Na^+,K^+-АТФазы. Активность Na^+,K^+-АТФазы увеличивалась в стриатуме и уменьшалась в стволе головного мозга.*

Выводы. *Продолжительное воздействие уабаина вызывает моторные нарушения, опосредованные изменениями активации сигнальных каскадов в нейронах дофаминергической системы.*

Ключевые слова: *Na^+,K^+-АТФаза; уабаин; кардиотонические стероиды; дофаминергическая система*

Этическое утверждение. Авторы подтверждают соблюдение институциональных и национальных стандартов по использованию лабораторных животных в соответствии с «Consensus Author Guidelines for Animal Use» (IAVES, 23.07.2010). Протокол исследования одобрен Этическим комитетом СПбГУ (протокол № 131-03-1 от 25.03.2019).

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Introduction

Cardiotonic steroids (CTS), also known as cardiac glycosides, are a group of compounds synthesized by some plants and animals. They bind to the α-subunit of Na^+/K^+-ATPase, reversibly inhibiting its activity. In its inhibited state, the Na^+/K^+-ATPase does not maintain and, in case of neurons, restore the resting membrane potential. The specific CTS binding site was found between M1-M2, M5-M6, and M7-M8 transmembrane domains of the α3 subunit, on the extracellular surface of the protein. CTS binding stabilizes the Na^+/K^+-ATPase complex in an E2P conformation, thereby reversibly inhibiting its pump activity [1]. Although high concentrations of CTS inhibit the Na^+/K^+-ATPase [2], at nanomolar concentrations they can cause an increase in its activity [3]. This effect is explained by the presence of tetrameric complexes of inactive Na^+/K^+-ATPase heterotetramers on the plasma membrane, which break down when one of the enzymes is bound by a CTS. The breakdown of these tetrameric complexes of Na^+/K^+-ATPase heterotetramers causes an increase in the amount of active enzymes [4]. In laboratory rodents, the α1 isoform of Na^+/K^+-ATPase is present in all cells and is insensitive to CTS, making these animals suitable for studying the effects of CTS specifically on the central nervous system (CNS) [5]. In the CNS, the α2 isoform (in astrocytes) and neuron-specific α3 isoforms of the Na^+/K^+-ATPase are present in addition to the ubiquitous α1 isoform [6].

Studies on rat neuron cultures showed that CTS concentrations that inhibit the Na^+/K^+-ATPase α3 isoform are neurotoxic [7], whereas concentrations that do not inhibit Na^+/K^+-ATPase activity are neuroprotective [8, 9]. Although toxic at higher concentrations, CTS are used as drugs: eg, digoxin derived from *Digitalis lanata* is used for heart failure treatment [10]. A number of studies suggest the presence of endogenous analogues of CTS in mammals [11]. However, it was found that patients can develop mood alterations [12] and sometimes delirium [13] when taking low concentrations of digoxin. This discovery led to the development of various bipolar disorder models based on intracerebroventricular (ICV) administration of ouabain to rats and mice [14–16].

Both ouabain (g-strophanthin) and digoxin are glycosylated cardenolides and are used to treat heart failure. In 2019, a mouse model of ouabain-induced bipolar disorder demonstrated that a single ICV administration of ouabain causes mania-like behavior, increased synthesis of dopamine, and reduced rate of dopamine reuptake from the synaptic cleft [16]. Thus, it was confirmed that

the effects observed following ouabain administration are mediated, among other factors, by changes in dopaminergic system functioning. The change in Akt protein kinase and ERK1/2 MAP kinase activation, associated with activation of dopamine D2 receptors and previously shown in rats, was also reproduced.

Patients with Parkinson disease (PD) were found to have an increased concentration of serum digoxin [17], indicating that endogenous analogues of CTS could potentially contribute to PD pathogenesis. Furthermore, neriifolin is used as the CTS to model PD in zebrafish (*Danio rerio*) [18]. Although the mechanisms behind the role of endogenous CTS in PD pathogenesis are yet to be determined, their role in the development of bipolar disorder and depression has been demonstrated in a number of studies [19].

The neurotoxicity of CTS is linked to oxidative stress and apoptosis mediated by increased ERK1/2 activation [20]. It should be noted that a similar mechanism can be observed in the 6-hydroxydopamine-induced model of parkinsonism. Based on the findings above, it could be hypothesized that long-term exposure to CTS may affect the function and viability of dopaminergic neurons via their indirect effect on the dopamine active transporter (DAT). It is presumed that chronic impairment of dopamine metabolism may be one of the mechanisms underlying dopaminergic neuron degeneration [21]. A number of studies indicate that such neuropsychiatric disorders as bipolar disorder, attention-deficit/hyperactivity disorder, and depression are risk factors for PD development. The dysfunction of dopamine receptors and altered dopamine reuptake plays a key role in the pathogenesis of these disorders [22–25]. An imbalance of these transporters' activity towards increased membrane DAT activity causes toxic dopamine metabolites to accumulate within the cytoplasm, leading to oxidative stress [26–28]. However, DAT dysfunction also has a negative impact on the neuron function. Partial loss of the DAT function can cause bipolar disorder, whereas complete DAT dysfunction results in juvenile parkinsonism [29]. Some researchers suggest measuring DAT activity in olfactory bulbs can be a prognostic factor of increased risk of PD development [30]. Previous studies have shown that DAT dysregulation (both decreased and increased DAT activity) can cause a number of pathological processes and may play a pivotal role in PD pathogenesis. Short-term inhibition of DAT does not significantly affect the risk of PD development, and the function of dopaminergic system is quickly restored [31, 32]. As such, the factors underlying long-term DAT dysfunction and, as a result, dopaminergic system dysfunction as a whole, lie beyond its immediate components. Exposure to such factors probably affects connecting links, dysfunction of which occurs due to the effect of factors that cause the death of dopaminergic neurons. One of such connecting links could be the Na^+/K^+-ATPase.

Studying disorders caused by long-term exposure of the dopaminergic system to low concentrations of CTS can help identify new mechanisms behind its dysfunction and regulation and improve our understanding of the role CTS play in the CNS.

Our **objective** was to study the effects of long-term ICV ouabain administration to C57Bl/6 mice on motor function, activity of dopamine-dependent signaling cascades, and ratios of apoptosis-regulatory proteins Bcl-2 in the striatum, as well as the impact of a single administration of ouabain on Na^+/K^+-ATPase activity in various brain structures.

Materials and methods

Study animals

We performed experiments on C57Bl/6 male mice 4–6 months old ($n = 40$) from the Saint Petersburg State University vivarium. The animals were housed in individually ventilated cages (temperature, 22 ± 1°C; relative humidity, 50–70%) with a 12-hour light/dark cycle (lights from 8 AM to 8 PM). The mice had *ad libitum* access to food and water. The mice were maintained according to the regulations governing the use of laboratory animals in research (as recommended by the Federation of European Laboratory Animal Science Associations and the Russian Laboratory Animal Science Association).

The experimental procedure was approved by the Animal Experimental Ethics Committee of Saint Petersburg State University (Protocol No. 131-03-1 dated March 25, 2019).

Substance administration

The animals were anesthetized with isoflurane (IsoFlo). Following the thorough cleaning of the skull from surface tissues, a guide cannula made of a 26G needle (KDF) and 1×2 mm fixed plastic holder [33] was unilaterally stereotaxically implanted using the following coordinates: AP (anteroposterior) = –0.5; L (lateral) = 1.0, to a depth of 2.0 mm so that the cannula tip was immediately above the lateral cerebral ventricle but did not protrude into it. The guide cannula was fixed with acrylic cement. Then a 3.9–4.0 mm long dummy cannula made out of a 33G needle (Mesoram) was inserted into the guide cannula to prevent its occlusion after the surgery. Experiments began 3 days after cannula implantation.

ICV injection was performed via an injection cannula, which was made out of a 33G needle connected to a Hamilton syringe. The cannula was inserted into the guide cannula to a depth of 2.5 mm. To ensure even injection, a syringe pump was used to deliver solu-

tions at 0.75 μL/min. The animals were administered 1.5 μL of ouabain dissolved in artificial cerebrospinal fluid (125 мМ NaCl, 26 мМ NaHCO3, 4 мМ KCl, 1.25 мМ NaH_2PO_4, 2 мМ $CaCl_2$, 2 мМ $MgCl_2$, 25 мМ glucose). The control group received 1.5 μL of artificial cerebrospinal fluid.

Behavioral testing

The open field test was used to assess locomotor activity and stereotypic behavior. The mouse was placed in the center of arena (40 × 40 × 40 cm). The distance traveled over the course of 20 minutes was recorded using the EthoVision XT video tracking software (Noldus). In EthoVision XT, the image of the square field was visually divided into zones: 4 corners, 4 edges, and the central zone. The central zone was a square in the field center. Its diagonal was equal to half of the entire field's diagonal. Motor stereotypy during the open field test was analyzed using an EthoVision XT algorithm. This algorithm calculates the index of spontaneous alterations: the number of alterations, Alt (the number of trajectory segments where the animal passed neighboring zones of the open field, except for the central one, in succession); the maximum number of alterations, mAlt (difference between the total number of zones the animals visited irregularly and the number of zones selected for analysis without one zone); index of alterations or index of stereotypy, IAlt (percentage of the number of alterations to the maximum number of alterations).

$I_{Alt} = Alt/mAlt \times 100\%$.

The beam balance test was used to evaluate motor coordination. The mice were placed with all 4 limbs at the beginning of a smooth circular wooden rod (diameter, 10 mm; length, 100 cm) elevated 80 cm above the floor. The animal's body was oriented along the rod. A flat rectangular platform (15 × 15 cm) was placed at the opposite end of the rod. The animals were trained to walk along the rod before the experiment. During the test, the total traversal time (from the beginning of the rod to the platform) and the number of paw slips (errors) and falls were recorded.

The ladder rung walking test assessed fine motor skills and motor coordination. The mice were placed at the beginning of a ladder with 2 mm diameter metal bars spaced 1.5 cm apart and inclined at an angle of 15°. The home cage was at the beginning of the ladder. The animals were trained to walk the ladder before the experiment. During the test, the walking time and the number of paw slips (errors) were recorded.

Material harvesting

The mice were euthanized by cervical dislocation. The brain was extracted on ice, dissected (striatum, brainstem, cerebellum, and hippocampus), and frozen in liquid nitrogen. The material was stored at −80ºC.

Determination of Na^+/K^+-ATPase activity

Na^+/K^+-ATPase activity was determined using inorganic phosphate accumulation. All procedures were performed on ice. The weighed tissue was homogenized using the Schuett Homgenplus homogenizer (SchuettBiotec GmbH) in 10-fold volumes of extraction buffer (0.25 M sucrose, 1 mM EDTA, 20 mM Tris, pH 7.45) that contained protease and phosphatase inhibitor cocktails (1 : 1000, Sigma) added immediately prior to use. The homogenate was centrifuged for 2 minutes at 4ºC and 1000g. The supernatant was transferred to a separate tube and centrifuged again for 15 minutes at 4ºC and 10 000g. The synaptosomal fraction was resuspended in isolation buffer and stored at −80ºC. A small volume of the sample was lysed with RIPA buffer (Sigma) that contains protease and phosphatase inhibitor cocktails, and the protein concentration was determined using DC Protein Assay Kit (Bio-Rad). The synaptosomal fraction with a protein concentration of 2 μg/μL was incubated with 0.065% sodium deoxycholate for 30 minutes in cold water bath. The obtained Na^+/K^+-ATPase preparation was added to the reaction medium (130 mM NaCl, 20 mM KCl, 3 mM $MgCl_2$, 30 mM imidazole, pH 7.5) to a final concentration of 0.05 μg/μL. A saturated solution of ouabain dissolved in the reaction buffer was used to measure the activity of other ATP-dependent enzymes. The reaction was started by adding 3 mM ATP, after which the reaction mixture was incubated for 15 minutes at 37ºC. The reaction was stopped by adding 0.1 mL of cooled 3 M acetate buffer (pH 4.3) containing 3.7% formaldehyde. To determine the amount of inorganic phosphate released, 0.02 mL of 2% ammonium heptamolybdate and 0.02 mL of freshly prepared 0.3% tin (II) chloride solution were added to the sample. The samples were thoroughly vortexed and incubated for 10 minutes. The optical density of the solution was measured at 735 nm using the Synergy H1 plate reader (BioTek). The Na^+/K^+-ATPase activity was calculated using the difference between the optical density in the test sample and that in the sample with the saturated ouabain solution.

Western blotting

Tissue samples were lysed in RIPA buffer (Sigma) with the addition of protease and phosphatase inhibitors (1 : 1000, Sigma). The resulting lysate was centrifuged at 14 000g at 4ºC for 20 minutes, then the supernatant was collected, and the protein concentration was measured using the DC Protein Assay Kit (Bio-Rad). Proteins were separated by polyacrylamide gel electrophoresis according to Laemmli. Then the proteins were transferred to a PVDF membrane (Whatman) and incubated with antibodies according to the manufacturers' in-

structions. For the analysis, we used primary antibodies to p-Akt (Ser473), Akt, p-ERK1/2 (Thr202/Tyr204), ERK1/2, Bak, Bax, Bcl-2, Bcl-xL (Cell Signaling Technology), pJNK (Thr183/Tyr185), JNK, NR2B, GAPDH and β-actin (Santa Cruz Biotechnology), NKA α1 a6F (DSHB), and NKA α3 (Thermo Scientific) and horseradish peroxidase-conjugated secondary anti-rabbit and anti-mouse antibodies (Cell Signaling Technology). The membranes were developed using SuperSignal West Femto Maximum Sensitivity Substrate (Thermo Scientific). Luminescence was detected using ChemiDoc MP Gel Documentation System (Bio-Rad), and its intensity was calculated using Image Lab 6.0.1 (Bio-Rad). Kinase activation was evaluated by the ratio between the intensity of the bands of the phosphorylated form of the kinase and the intensity of the bands of its total form (phosphorylation level).

Statistical analysis

Statistical analysis was performed using the GraphPad Prism 7 software. Data are presented as arithmetic mean ± standard error of the mean (mean ± SEM). Data were analyzed using *t* test and 1-way or 2-way ANOVA with Tukey test and preliminary Shapiro–Wilk test. Differences were considered significant at $p < 0.05$.

Results

Motor dysfunction and locomotor activity in mice after ouabain injection

The effect of ICV injection of 1.5 μL of 50 μM (75 pmol, 43.8 ng) ouabain on the neurological status and locomotor activity of the animals was evaluated 24 hours after 4-day ouabain administration. To assess the neurological status of the animals, the beam and ladder rung walking tests were used.

The ladder rung walking test performed at 24 hours after 4-day ICV administration in ouabain-treated mice took 1.6 times longer ($p = 0.045$) with 3 times more errors ($p < 0.028$) compared with the control animals (Fig. 1, *A*). On day 5, the beam balance test duration in the ouabain-treated group doubled ($p < 0.031$) with a 3.6-fold increase in the number of errors ($p < 0.02$)

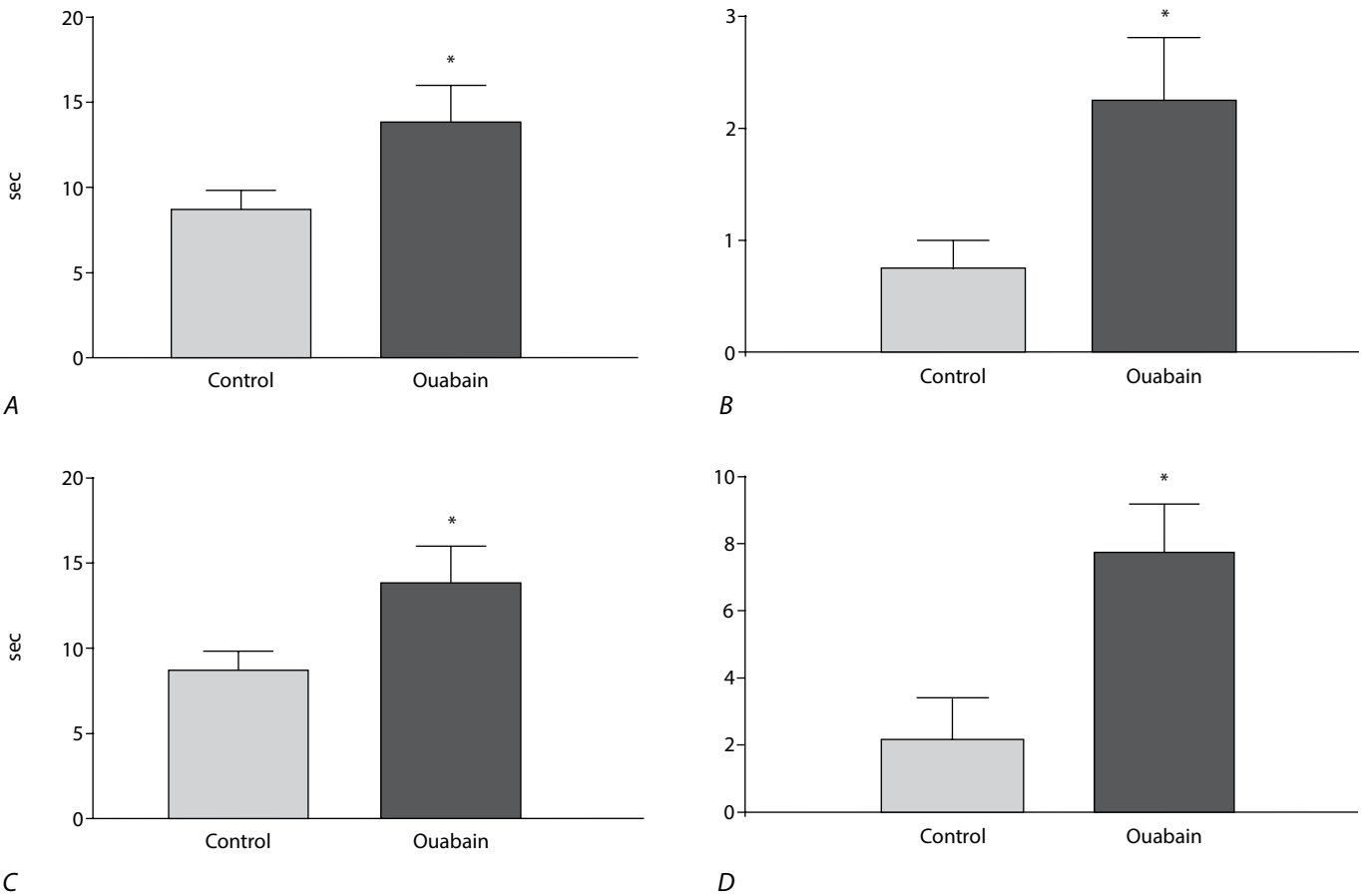

Fig. 1. The effect of 4-day ICV ouabain administration to C57Bl/6 mice ($n = 5$) on the traversal time (*A*), the number of errors in ladder rung walking test (*B*), distance (*C*), the number of errors in the beam balance test (*D*).
The data are presented as mean ± SEM; *$p < 0.05$.

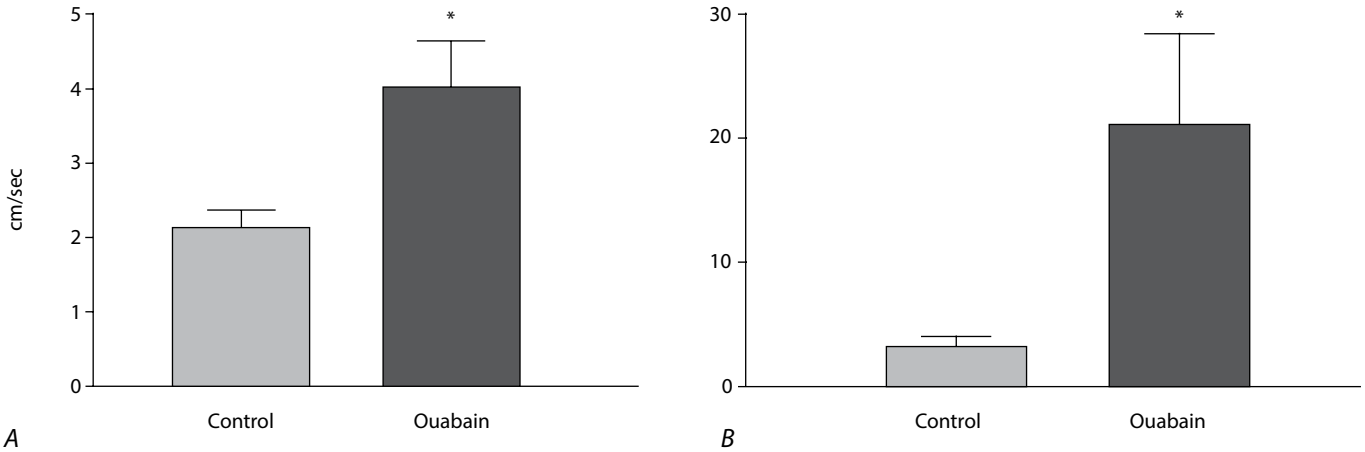

Fig. 2. The effect of 4-day ICV ouabain administration to C57Bl/6 mice ($n = 5$) on motor activity (*A*) and IAlt (*B*) values acquired from the 20-minute open field test.
The data are presented as mean ± SEM; *$p < 0.05$.

compared with the controls. Based on the data presented, it could be hypothesized that multiple injections of ouabain in C57Bl/6 mice result in disrupted ability of keeping body balance and fine motor impairments.

To assess locomotor activity 24 hours after 4-day ouabain administration, a 20-minute open field test was performed where the motor function of the animals was evaluated based on the average walking speed. To assess stereotypic behavior, we calculated IAlt values.

The mice motor activity expressed as average walking speed at 24 hours after 4-day ICV ouabain administration showed a 1.9-fold increase ($p = 0.029$) in comparison with the controls (Fig. 2, *A*). The IAlt value in the open field test performed 24 hours after 4-day ouabain administration in the ouabain group was 7 times higher than that in the control group ($p = 0.036$; Fig. 2, *C*), which means that stereotyped behavior in the ouabain-treated mice was more manifested. Based on the data presented, we may suggest that multiple ouabain injections result in the increased motor activity and stereotyped behaviors persisting on day 1 after the last injection, also with ataxia development.

Effects of ouabain on catalytic activity of the Na^+/K^+-ATPase in the mouse brain

To evaluate the effects of ouabain on the CNS of C57Bl/6 mice, it is necessary to determine how the dose administered via ICV affects the catalytic activity of the Na^+/K^+-ATPase in various parts of the animal brain. Na^+/K^+-ATPase activity was measured in a crude synaptosomal fraction of the striatum, hippocampus, brainstem, and cerebellum in the control group and 10 and 30 minutes after ouabain injection.

The Na^+/K^+-ATPase activity measured at 10 minutes after ouabain injection in the synaptosomal fraction of the animal striatum showed a 1.4-fold increase ($p < 0.05$). There was no significant difference in activity levels at 30 minutes after the injection compared with the control group (Fig. 3, *A*). 5 mM ouabain-resistant Na^+/K^+-ATPase activity was 4.1 μM Pi/mg protein/min.

Na^+/K^+-ATPase activity in the synaptosomal fraction derived from the hippocampus at 10 and 30 minutes after ouabain injection was not significantly different from the control group (Fig. 3, *B*). The enzyme activity resistant to inhibition by 5 Mm ouabain was 4.1 μM Pi/mg protein/min.

Activity of Na^+/K^+-ATPase in the synaptosomal fraction derived from the animal brainstem measured at 10 minutes after ouabain administration was not significantly different from the control. Activity level measured at 30 minutes after the administration showed a 1.8-fold decrease (Fig. 3, *C*; $p < 0.05$). The enzyme activity resistant to inhibition by 5 Mm ouabain was 5 μM Pi/mg protein/min.

Based on the data obtained, we may conclude that ICV injection of 1.5 μL of 50 μM ouabain into the lateral cerebral ventricle results in a short-term increase of Na^+/K^+-ATPase activity levels in striatum at 10 minutes after the injection and a decrease in enzyme activity in the animal brainstem at 30 minutes after the injection.

Effects of ouabain on activation of intracellular signaling kinases and on protein content in mouse striatum

Earlier studies show that a single ICV injection of 50 μM ouabain induces Akt and ERK1/2 activation [16]. Primary cerebral cortex neuron culture assays proved that long-term exposure to ouabain (6–18 h) induces the inactivation of another MAP-kinase: JNK [7]. To find out the effects of multiple ICV ouabain injections on the activation of intracellular signaling pathways associ-

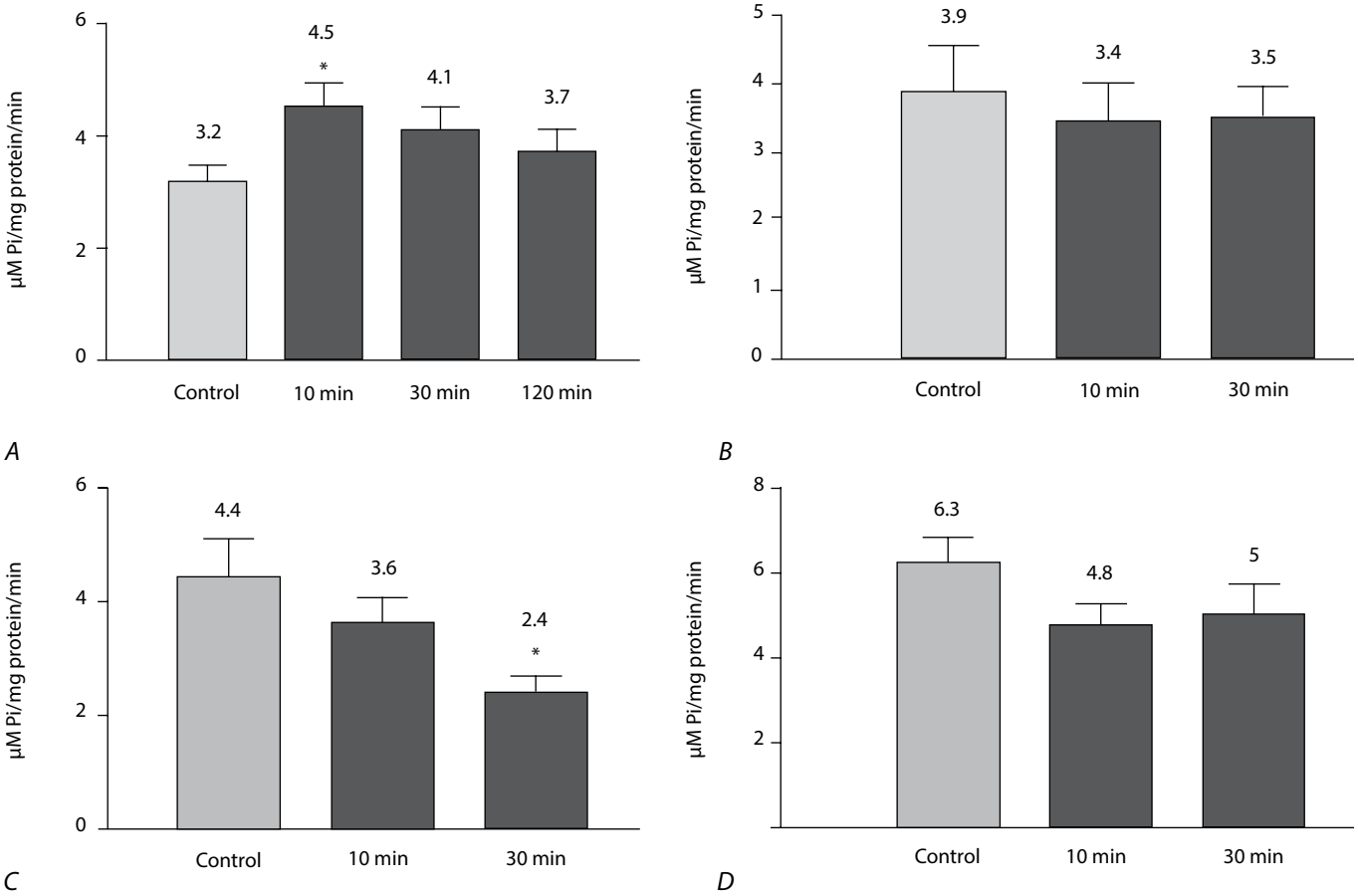

Fig. 3. The ouabain effects on Na^+/K^+-ATPase activity in the synaptosomal fraction derived from the striatum (*A*), hippocampus (*B*), brainstem (*C*), and cerebellum (*D*) in C57Bl/6 mice ($n = 5$) at 10 and 30 minutes after the ICV injection into a lateral cerebral ventricle.
The data are presented as mean ± SEM; *$p < 0.05$.

ated with dopamine receptors and with earlier-studied effects of ouabain, the activation of Akt, ERK1/2 and JNK kinases after once daily 4-day administration of 50 µM ouabain in the animal striatum was evaluated. The analyses were performed on day 5 and 24 hours after the last ouabain administration. Activation of the kinases was evaluated by comparing the ratio of phosphorylated kinase level to the level of its nonactivated form in the controls and the ouabain-treated animals using Western blotting.

Once daily 4-day 50 µM ouabain administration resulted in a decrease of Akt activationin the animal striatum ($p < 0.05$) measured 24 hours after the last injection by 62.2% compared with the control group (Fig. 4, *A*). At the same time, ERK1/2 and JNK activationcompared to the controls did not change (Fig. 4, *B*, *C*). Based on the data obtained, we can conclude that the long-term effects of ouabain do not include alterations in ERK1/2 and JNK MAP kinases activation. Still, it is possible to assume that, as a result of the treatment, intracellular signaling pathways switch to a "slow" response to activation of dopamine receptors leading to Akt kinase inactivation [34].

Data obtained from the primary rat neuron cultures show that ouabain can reduce the levels of NMDA-receptor subunits (NR2B) [35]. It was hypothesized that multiple ouabain injections may alter Na^+/K^+-ATPase levels along with the levels of NMDA-receptor interacting with this enzyme. To check this hypothesis, we evaluated the impact of ICV ouabain injection on the levels of α1 and α3 isoforms in Na^+/K^+-ATPase α subunit and on the levels of NR2B subunits in NMDA-receptors in the striatum of mice.

Multiple injections of ouabain did not affect the levels of α1 and α3 isoforms in Na^+/K^+-ATPase α subunit (Fig. 5, *A*, *B*). NR2B subunit levels in NMDA-receptors in the mouse striatum measured in the ouabain group 24 hours after the last injection decreased by 37.4% ($P < .05$) compared with the controls (Fig. 5, *C*).

Although according to the data obtained earlier, a single ICV injection of 50 µM ouabain does not induce neuronal apoptosis [16], it was hypothesized that multiple injections of 50 µM ouabain may result in disrupted homeostasis of the proteins regulating the mitochondri-

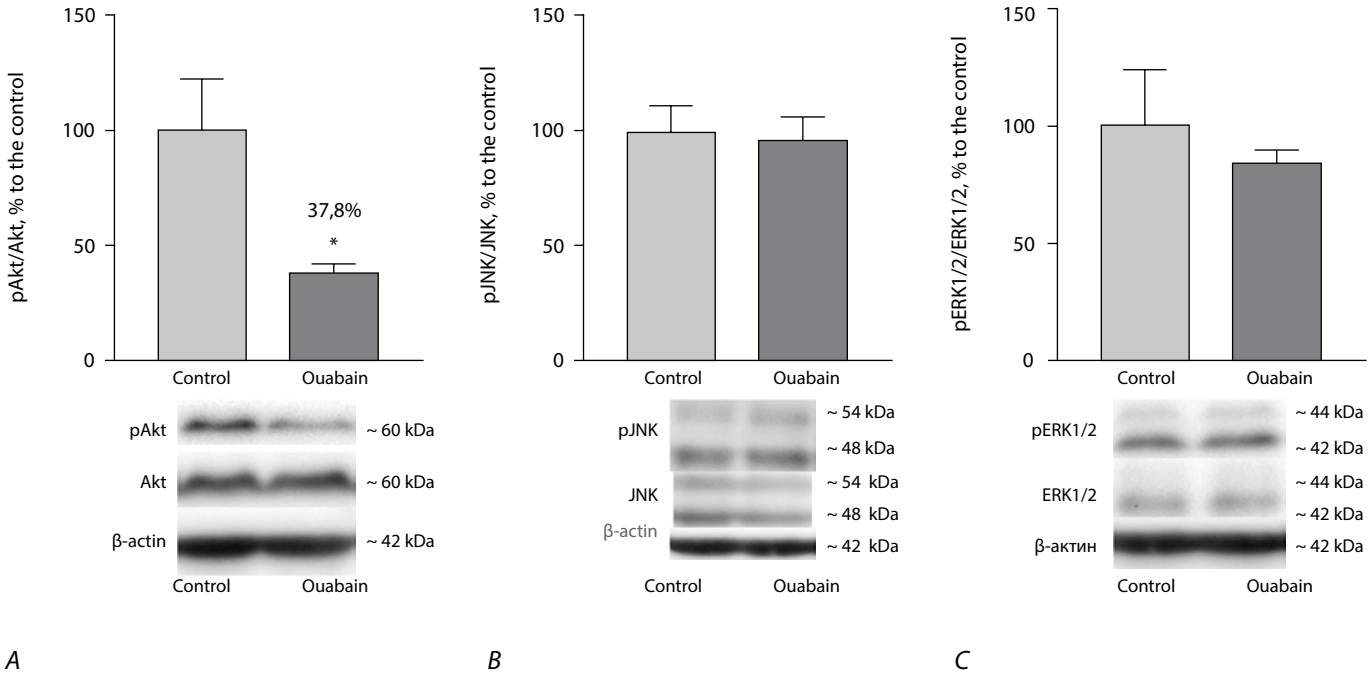

Fig. 4. The effects of once daily 4-day administration of 50 μM ouabain on Akt (*A*), ERK1/2 (*B*), and JNK (*C*) activation in the C57Bl/6 mice striatum measured 24 hours after the last injection.
The data are presented as mean ± SEM; *$p < 0.05$. Under the charts representative immunoreactive bands are presented.

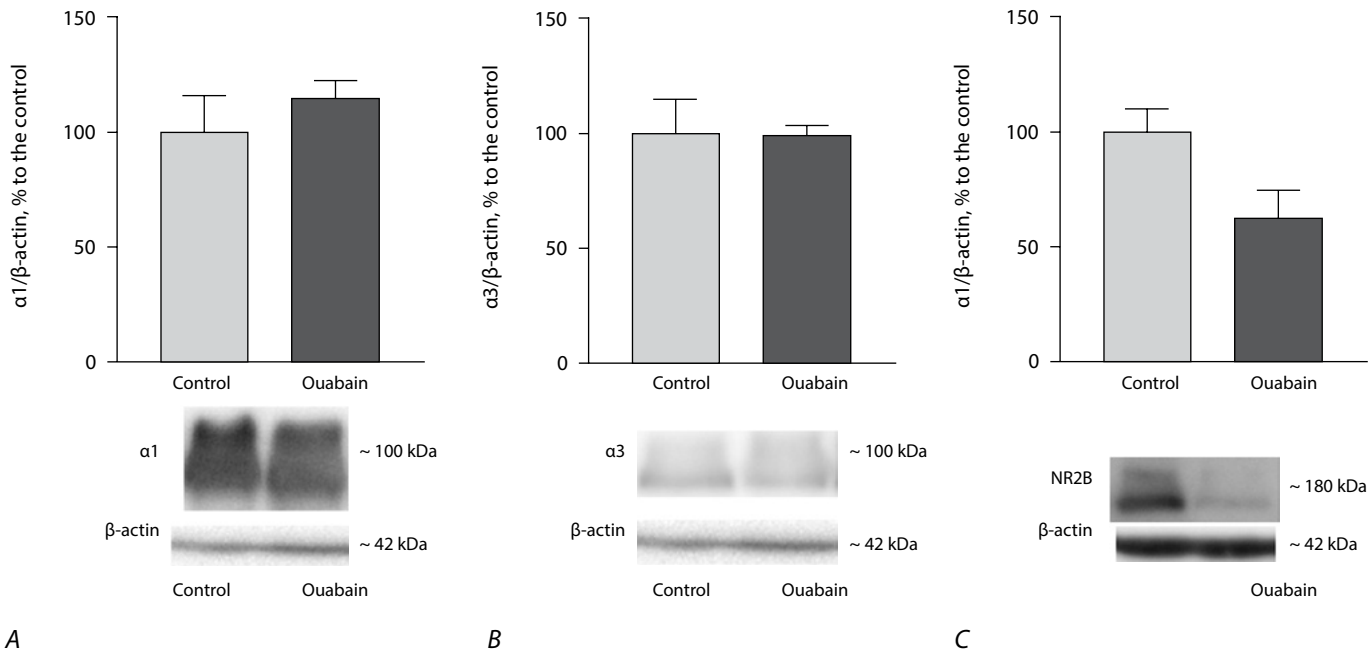

Fig. 5. The effect of once daily 4-day administration of 50 μM ouabain on the levels of α1 (*A*) and α3 (*B*) isoforms in α subunit of Na^+/K^+-ATPase and the levels of NR2B subunits in NMDA-receptors (*C*) in the mice's striatum measured 24 hours after the last injection.
The data are presented as mean ± SEM; *$p < 0.05$. Under the charts representative immunoreactive bands are presented.

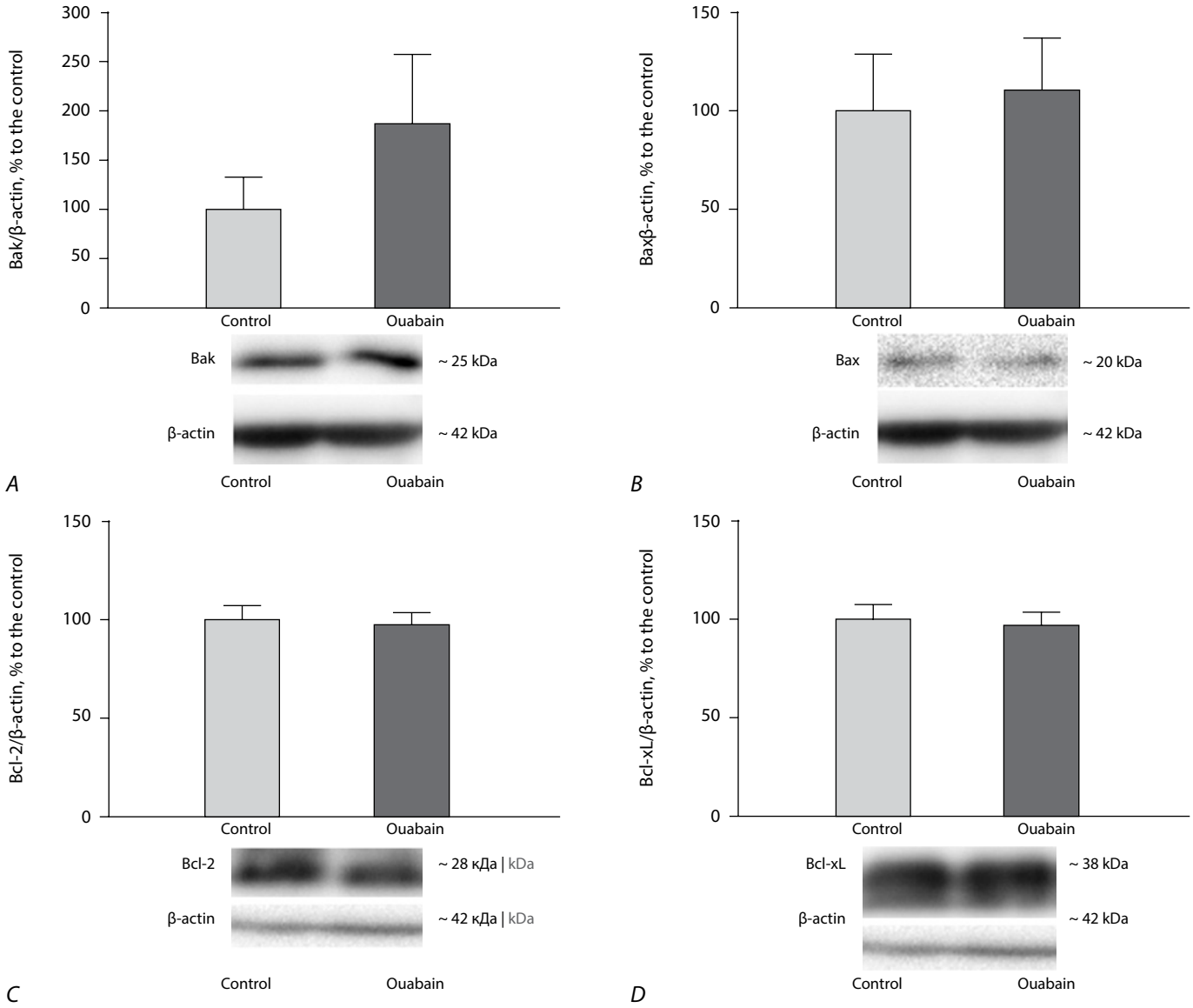

Fig. 6. The effects of once-daily 4-day administration of 50 μM ouabain on the levels of Bak (*A*), Bax (*B*), Bcl-2 (*C*), Bcl-xL (*D*) proteins in the C57Bl/6 mouse striatum measured 24 hours after the last injection.
The data are presented as mean ± SEM; **p* < 0.05. Under the charts representative immunoreactive bands are presented.

al pathway of apoptosis. To check this hypothesis, we studied the effect of once-daily 4-day administration of 50 μM ouabain on the levels of Bcl-2 family proapoptotic and antiapoptotic proteins in the striatum at 24 hours after the last injection.

As shown in Fig. 6, once-daily 4-day administration of 50 μM ouabain does not affect the levels of Bak, Bax, Bcl-2, and Bcl-xL proteins in the striatum, measured 24 hours after the last injection, compared with the controls. Thus, we can conclude that either ouabain appears to lack neurotoxicity for the striatum within the given experiment design, or its neurotoxicity is not associated with the alteration of the principal Bcl-2 family proteins regulating the mitochondrial pathway of apoptosis.

Discussion

To assess the body balance and fine motor skills, the ladder rung walking test and the beam balance test were used. In both tests the ouabain-treated animals made significantly more errors and needed more time to complete the tests compared with the controls. These findings may indicate that long-term administration of ouabain may impair balance and fine motor skills. These tests are used to assess a wide range of motor dysfunctions in PD animal models [36, 37]. A single ouabain injection in rodents has been already shown to result in mania-like behavior [16]. However, these kinds of deficits were not observed in animal models of mania [38], which suggests the occurrence of function-

al or organic deficits in the dopaminergic system in response to multiple injections of ouabain in a non-toxic concentration.

As a single ouabain injection resulted in an increase of both motor activity and stereotypic behaviors induced by the activation of D2-mediated intracellular signaling pathways [16], we studied the effect on fine motor skills and motor function of the animals, and on activation of dopamine-mediated intracellular signaling pathways in 4-day ICV ouabain treatment. The motor activity and stereotypic behavior were evaluated in the open field test 24 hours after the last ouabain administration, and were more pronounced than the corresponding criteria in the control group. These effects did not emerge after a single ouabain injection [16].

There are studies indicating that lower concentrations of CTS can cause increased Na^+/K^+-ATPase activity. [3,4] It has been shown that the Na^+/K^+-ATPase is present in inactive tetrameric complexes on the cell membrane and ouabain binding to one of the enzymes promotes the breakdown of this complex [4]. The breakdown of the tetrameric complex results in enzyme release and activation, increasing the total effective Na^+/K^+-ATPase activity in the cell. The increase of effective Na^+/K^+-ATPase activity in striatum registered at 10 minutes after ICV ouabain injection suggests the penetration of a low ouabain dose into the striatum causing alterations in the signaling function of the Na^+/K^+-ATPase, without reducing its activity. Increase of enzyme activity might be mediated by other mechanisms, such as protein recruitment into the cell membrane. On the contrary, decreased enzyme activity in the brainstem observed at 30 minutes after the injection implies higher ouabain concentrations in this part of the brain. However, it is impossible to specify which concentrations of CTS reach certain parts of the brain without measuring its levels in the brain samples using mass spectrometry. It could be suggested that similar effects in the human brain would appear with substantially lower CTS concentrations than the ones used in this experiment because the Na^+/K^+-ATPase α subunit α1, α2, and α3 isoforms in humans are by 1-2 orders of magnitude more sensitive to ouabain than in mice (based on the results obtained in various studies) [39, 40].

Based on increased Na^+/K^+-ATPase activity in the striatum, we can assume that ouabain reaches this part of the brain in low concentrations following administration, and affects the Na^+/K^+-ATPase signaling function. It is known that mania-like behavior in mice induced by a single ouabain injection is associated with ERK 1/2 and Akt kinase activation [16] and that activation of these pathways is mediated by D2 dopamine receptors. We can suggest that decreased Akt activation followed by GSK3β-kinase activation occurs due to the activation of a slower pathway regulated by β-arrestin [34].

Higher concentrations of CTS can cause nonspecific cell death by activating apoptotic signaling pathways. [41] To exclude this process, we assessed the antiapoptotic (Bcl-2, Bcl-xL) and proapoptotic (Bak, Bax) protein levels in the mouse striatum. The absence of significant alterations in the protein levels indicates that CTS concentrations used in this study did not activate the mitochondrial apoptosis pathway.

The reduced levels of the NMDA-receptor NR2B subunit in the mouse striatum after 4-day administration of 50 μM ouabain correspond to the data obtained earlier using primary cultures of rat cerebellum cells [35]. This effect may explain earlier findings made by other researchers, which demonstrated an ouabain-induced decline in spatial memory in rats [15], because NR2B subunits are known to play an important role in memory formation [42, 43]. The lack of change in Na^+/K^+-ATPase α1 and α3 isoform levels indicates that the long-term physiological effects of ouabain are not associated with alterations in total Na^+/K^+-ATPase quantity.

Conclusion

Once daily 4-day aadministration of 50 μM ouabain in C57Bl/6 mice causes hyperlocomotion which persists on day 5 of the study and is associated with impaired motor coordination. The behavior alterations observed are caused by altered dopaminergic transmission, presumably accompanied by the activation of a slower β-arrestin pathway and by reduced Na^+/K^+-ATPase activity in the brainstem. At the same time, Na^+/K^+-ATPase activity increases in the striatum.

The results presented in this study show that long-term exposure of the CNS Na^+/K^+-ATPase to CTS causes dopaminergic system dysfunction, and suggest the possibility of managing dopaminergic disorders via pharmacological regulation of the Na^+/K^+-ATPase.

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Copper Ions Reduced Toxicity of Sodium Azide and Lipopolysaccharide on Cultured Cerebellar Granule Neurons

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Abstract

Introduction. *Copper ions (Cu^{2+}) are structural elements of proteins such as cytochrome c oxidase (Complex IV), an enzyme that catalyzes the final step of electron transfer to oxygen during oxidative phosphorylation in the mitochondria. With Cu^{2+} homeostasis being of utmost importance, its disturbances in the central nervous system are involved in the mechanisms of many neurodegenerative and other brain disorders.*

This study aimed *to assess the effects of non-toxic copper ion levels on death of cultured cerebellar granule neurons associated with lipopolysaccharide (LPS; in vitro inflammation model) or azide sodium (NaN_3; cytochrome c oxidase inhibitor).*

Materials and methods. *LPS (10 μg/mL) or NaN_3 (250 μM) was added on day 7 to 8 to the culture medium with rat cerebellar cells for 24 hours in vitro. Nitrite concentrations were measured in the culture medium by Griess assay; absorbance was recorded with a spectrophotometer at 540 nm, and morphologically intact cells were counted as survived neurons.*

Results. *Added to the culture medium, LPS or NaN_3 reduced neuron survival to 15 ± 2% or 20 ± 3% vs. control, respectively. Cu^{2+} (0.5 to 5.0 μM) increased neuron survival in a dose-dependent manner to 78 ± 4% with toxic levels of LPS and to 86 ± 6% with NaN3 with 5 μM Cu^{2+}. The concentration of nitrites in the control culture medium was 2.0 ± 0.2 μM. Added to the cell cultures, LPS increased the concentration of nitrites to 8.5 ± 0.5 μM. Cu^{2+} 5 μM did not show any significant effects on nitrite accumulation in the culture medium.*

Conclusions. *We showed that copper ions can exert protective effects on neurons against LPS-induced or NaN_3-induced toxicity. This protection is likely to be associated rather with Cu^{2+} interaction with Complex IV of the electron transfer chain in the mitochondria than with inhibition of NO production. Effects of Cu^{2+} on apoptosis pathway proteins also cannot be ruled out.*

Keywords: *neurons; copper ions; sodium azide; nitrogen oxide*

Ethics approval. Authors confirm compliance with institutional and national standards for the use of laboratory animals in accordance with «Consensus Author Guidelines for Animal Use» (IAVES, 23 July 2010). The research protocol was approved by the Local Ethics Committee of the Research Center of Neurology (protocol No. 5-5/22, June 1, 2022).

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Ионы меди снижают токсическое действие азида натрия и липополисахарида на культивированные зернистые нейроны мозжечка

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Аннотация

Введение. *Ионы меди (Cu^{2+}) являются структурными элементами белков, в том числе цитохром с-оксидазы (комплекс IV) — фермента, катализирующего конечный этап переноса электронов на кислород в процессе окислительного фосфорилирования в митохондриях. Поддержание гомеостаза Cu^{2+} в головном мозге очень важно, и его нарушение в центральной нервной системе вовлечено в патогенез многих нейродегенеративных заболеваний и патологических состояний головного мозга.*

Цель *исследования — определить влияние нетоксических концентраций ионов меди на гибель культивированных зернистых нейронов мозжечка, вызванную липополисахаридом (ЛПС; модель воспаления in vitro) и азидом натрия (NaN_3, ингибитор цитохром с-оксидазы).*

Материалы и методы. *ЛПС (10 мкг/мл) или NaN_3 (250 мкМ) добавляли на 7–8-й день in vitro в среду культивирования клеток мозжечка крыс на 24 ч. Уровень нитритов измеряли в среде культивирования методом Грисса, оптическую плотность регистрировали при длине волны 540 нм с помощью спектрофотометра, а число живых нейронов оценивали методом подсчёта морфологически интактных клеток.*

Результаты. *Добавление в среду культивирования ЛПС снижало выживаемость нейронов до 15 ± 2% относительно контроля, а NaN_3 — до 20 ± 3%. В присутствии Cu^{2+} (0,5–5,0 мкМ) выживаемость нейронов дозозависимо повышалась: на фоне 5 мкМ Cu^{2+} при токсическом воздействии ЛПС — до 78 ± 4%, а при действии NaN_3 — до 86 ± 6%. В среде культивирования контрольных культур содержание нитритов составляло 2,0 ± 0,2 мкМ. Добавление ЛПС вызывало повышение уровня нитритов до 8,5 ± 0,5 мкМ. Ионы меди не оказывали достоверного влияния на накопление нитритов в среде культивирования.*

Заключение. *Показана возможность защитного действия ионов меди на нейроны при токсичности, вызванной ЛПС и NaN_3. Видимо, эта защита обусловлена взаимодействием Cu^{2+} с комплексом IV цепи переноса электронов в митохондриях, а не подавлением продукции оксида азота, не исключено также влияние Cu^{2+} на белки путей апоптоза.*

Ключевые слова: *нейроны; ионы меди; азид натрия; оксид азота*

Этическое утверждение. Авторы подтверждают соблюдение институциональных и национальных стандартов по использованию лабораторных животных в соответствии с «Consensus Author Guidelines for Animal Use» (IAVES, 23.07.2010). Протокол исследования одобрен Локальным этическим комитетом ФГБНУ НЦН (протокол № 5-5/22 от 01.06.2022).

Источник финансирования. Авторы заявляют об отсутствии внешних источников финансирования при проведении исследования.

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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Introduction

Copper is one of the most abundant transition metals in the human body. It takes part in oxygen metabolism, collagen synthesis, and skin pigmentation, maintaining the integrity of blood vessels, as well as in iron homeostasis, antioxidant defense, and neurotransmitter synthesis [1]. Cu^{2+} ions are structural elements of several proteins. For instance, copper is an essential component of cytochrome c oxidase (Complex IV), an enzyme that catalyzes the final step of electron transfer to oxygen during oxidative phosphorylation in the mitochondria. Copper ions are also contained in the superoxide dismutase molecule, which is the most important antioxidant, and ceruloplasmin, a blood plasma protein involved in the mechanisms of pro-oxidant and antioxidant reactions. Copper is also necessary for several important processes in the brain tissue, such as the regulation of intracellular signal transduction, catecholamine balance, myelination of neuron axons, and synaptic transmission in the central nervous system (CNS) [2].

The copper content in the brain ranges from approximately 3 to 5 μg/g wet weight [1]. Recommended copper intake to maintain systemic homeostasis in adults is 0.8 to 2.4 mg/day [3]. Stable Cu^{2+} homeostasis in the brain is essential, and its disturbances can be fatal for neurons. Cu^{2+} homeostasis disorders in the CNS are involved in the mechanisms of many neurodegenerative and other brain disorders, such as Wilson disease and Alzheimer's disease [4–6].

Intracellular copper and iron imbalance can increase free radical production and oxidative stress [78] because these transition metals directly participate in the Fenton reaction, which results in hydroxyl radicals with high toxicity [9]. Two-valent copper can mediate generation of hydrogen peroxide by tau-protein [9] and increase effects of pro-oxidants. Micromolar concentrations of the antioxidant acetylcysteine in the culture medium showed pro-oxidant activity with nanomolar concentrations of copper [10]. However, very limited literature data are available on direct effects of these ions on key neurodegeneration processes, including inflammatory processes in the CNS and mitochondria inhibition.

This study aimed to assess the effects of non-toxic Cu^{2+} levels on death of cultivated cerebellar granule neurons induced by lipopolysaccharide (LPS; *in vitro* inflammation model) or azide sodium (NaN_3; cytochrome c oxidase inhibitor).

Materials and methods

In our experiments, we used 7-day to 8-day cultures of the cerebellum from 8-day-old rats obtained by enzymatic and mechanical dissociation: 15 minutes at 36.5ºC in trypsin (0.05%) and EDTA (0.02%) solution in phosphate buffer (Gibco Life Technologies) followed by stepwise pipetting in the medium [10]. The cultures were cultured in 96-well plastic plates (Eppendorf) coated with poly-lysine (Sigma). The culture medium contained 90% minimum essential medium with Earle's salts (Gibco), 10% fetal bovine serum (HyClone), 2 mM glutamine (glutaMAX, Gibco), 25 μM KCl, and 10 mM HEPES buffer pH 7.2 to 7.4 (VWR Life Science). To each plate well, 0.1 mL of cell suspension was added to obtain a final cell density of 3 to 5 × 10_3 cells/mm^2. The cultures were developed in a CO_2-incubator at 36.5ºC and relative humidity 98%.

On day 7 to 8, copper (II) chloride (0.5 to 5.0 μM, Sigma) with LPS (10 μg/mL, Sigma) or NaN_3 (250 μM) was added to the culture medium with 7-day rat cerebellar cells *in vitro* for 24 hours.

After the experiment, the cultures were fixed in ethanol + formaldehyde + acetic acid mixture (7 : 2 : 1) and stained with trypan blue. The cultures were photographed with an Olympus CKX41 inverted microscope or an EVOS M7000 imaging system (Termo Fisher Scientific) at × 40 objective magnification. Percentage of survived neurons was evaluated by counting morphologically intact cultured granule neurons in 5 consecutive field views. Survival in test cultures was expressed in per cent *vs*. control.

The level of nitric oxide (NO) was determined by Griess assay, which is based on the formation of diazo compounds that react with alpha-naphthylamine to give a red solution. Photometry was performed with a microplate reader (SpectraMax M2, Molecular Devices) at 540 nm.

Data were statistically processed with Statistica v. 13.3 (StatSoft Inc.) and one-way ANOVA with Newman–Keuls post hoc test or t-test. Between-group differences were considered statistically significant if $p < 0.05$. Results were presented as mean ± standard error of mean ($M \pm SEM$).

All procedures performed in the experiments involving animals complied with the ethical standards approved by the Russian regulations, the principles of the Basel Declaration, and Recommendations of the Local Ethical Committee of Research Center of Neurology (Protocol 5-5/22 of 1 June 2022).

Results

Cu^{2+} toxicity in cultured cells was seen with concentrations of at least 25 μM. With further increase in Cu^{2+} concentrations, neuron survival decreased in a dose-dependent manner (Fig. 1). Added to the culture medium, LPS or NaN_3 decreased neuron survival

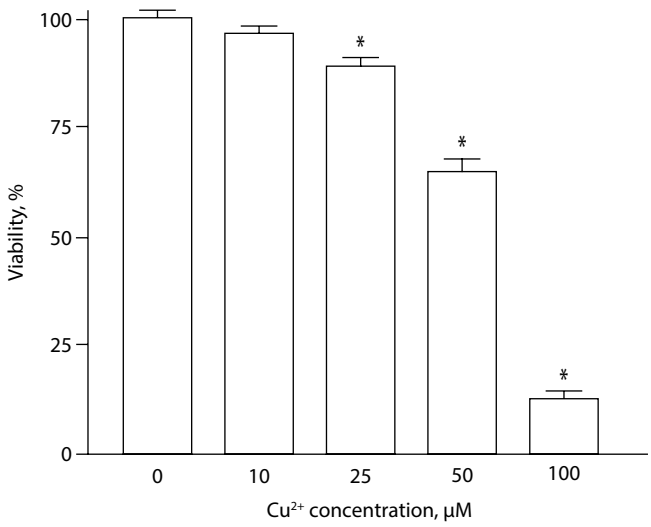

Fig. 1. Effects of different copper ion levels on survival of cultured rat cerebellar granule neurons.
*$p < 0.05$ vs. control (0 μM).

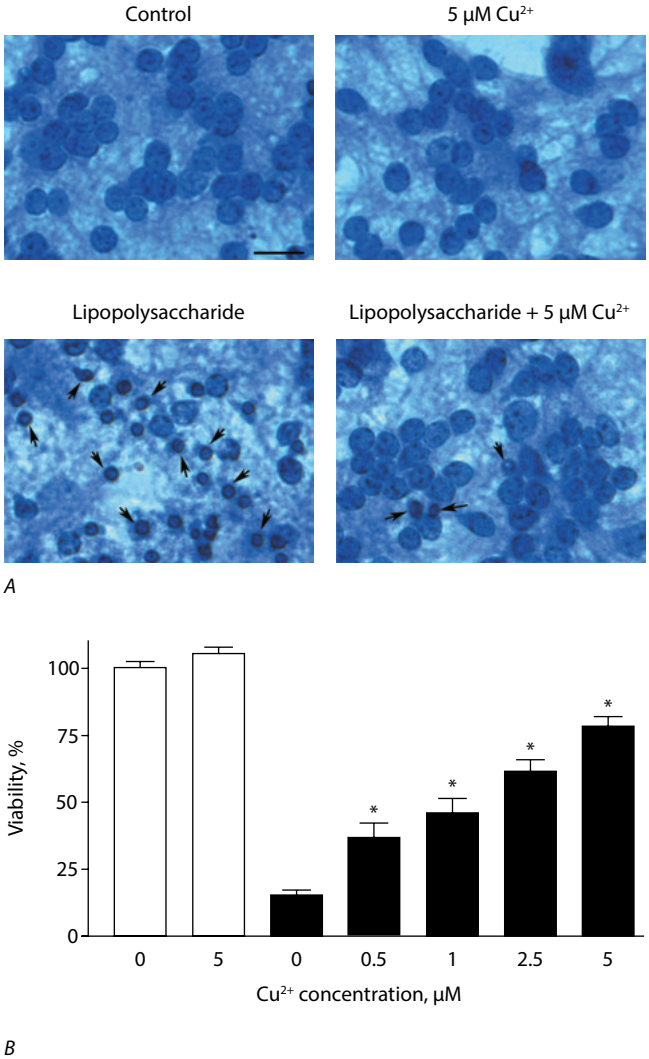

Fig. 2. Copper ions reduced LPS toxicity in cultured rat cerebellar granule neurons.
A: fixed cultures stained with trypan blue. Dead neuron nuclei are shown with arrows. Scale 15 μm.
B: quantitative data obtained by counting morphologically intact neurons without (white bars) and with LPS (black bars).
*$p < 0.05$ compared to 0 μM Cu^{2+} with LPS.

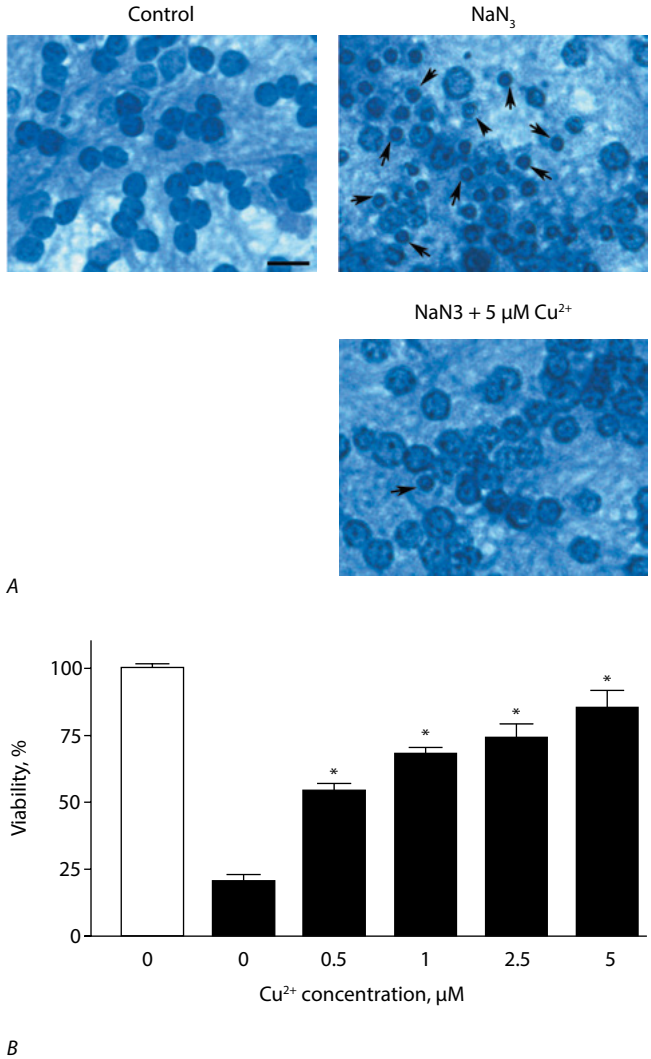

Fig. 3. Copper ions reduce NaN_3 toxicity in cultured rat cerebellar granule neurons.
A: fixed cultures stained with trypan blue. Dead neuron nuclei are shown with arrows. Scale 15 μm.
B: quantitative data obtained by counting morphologically intact neurons without (white bars) and with NaN_3 (black bars).
*$p < 0.05$ compared to 0 μM Cu^{2+} with NaN_3.

to 15 ± 2% (Fig. 2) or 20.0 ± 2.5% (Fig. 3) vs. control, respectively. If neurons were treated with the toxins in the presence of non-toxic copper ion levels, neuron survival increased in a dose-dependent manner.

Cu^{2+} 5 μM improved neuron survival to 78±4% in the experiment with LPS (Fig. 2) or to 86 ± 6% in the experiment with NaN3 (Fig. 3). The concentration of nitrites in the control culture medium was 2.0 ± 0.2 μM. Added to the cell cultures, LPS increased the concentration of nitrites to 8.5 ± 0.5 μM (Fig. 4). Copper chloride 5 μM did not have any significant effects on nitrite accumulation in the culture medium treated with LPS (Fig. 4).

Discussion

Imbalances of several metal ions, especially zinc and copper, are thought to play an important role in the pathogenesis of many neurodegenerative disorders, including multiple system atrophy, amyotrophic lateral sclerosis, Creutzfeldt–Jakob disease, Wilson disease, Alzheimer's disease, and Parkinson's disease [1, 11, 12]. Normally, copper ions are structural elements of many proteins, including ceruloplasmin, a blood plasma protein that is involved in the mechanisms of various prooxidant and antioxidant reactions. Copper is necessary for functioning of the antioxidant cell system because it is contained in the superoxide dismutase molecule. Cu

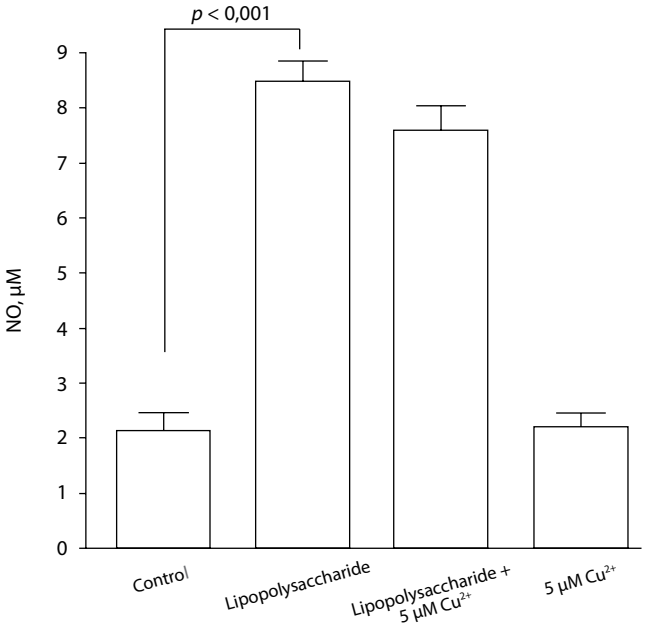

Fig. 4. The levels of nitrites (NO) in the culture medium of rat cerebellar granule neurons.
The addition of LPS (10 μg/ml, 24 h) causes an increase in nitrites in the culture medium. Cu^{2+} (5 μM) have no significant effect on the accumulation of nitrite in the culture medium under LPS action.

(II) derivatives are effective anti-inflammatory agents [13, 14], and Cu-binding peptides showed anti-inflammatory effects in primary microglia cultures [15].

NO is a key inflammation mediator. Glia cells with inflammatory activation, which is seen in most CNS disorders, were previously shown to be capable of exerting neuronal toxicity, which was prevented by inhibitors of inducible NO synthase [16]. Excessive formation of NO or reactive NO species, such as peroxynitrite, impairs mitochondrial functioning and eventually affects neuronal cell metabolism and survival [17, 18]. Besides its multiple regulatory functions, NO was found to modulate cell respiration by irreversibly inhibiting the cytochrome c oxidase activity [19, 20].

In our study we showed that LPS, which was added to the culture medium with neuroglia cultures, reduced survival of cultured rat cerebellar granule neurons and was associated with nitrite accumulation in the culture medium due to NO production. Added to the culture medium, non-toxic concentrations of Cu^{2+} significantly reduced LPS-induced cell death. NO is known to act as a ligand for copper atoms and cause a redox reaction with the metal after its binding. Furthermore, NO possesses an unpaired electron, which can couple with the unpaired electron on Cu^{2+} [21]. In our experiments, copper did not show any significant effects on nitrite accumulation in the culture medium treated with LPS. Moreover, NO can inhibit mitochondrial respiration mainly by competitively inhibiting oxygen binding by Cu^{2+}-containing cytochrome c oxidase (Complex IV) [22] and direct interaction of Cu^{2+} with tricarboxylic acid cycle enzymes [23]. Our experiments demonstrated that copper ions protected neurons against the toxicity induced by NaN_3, which inhibits Complex IV of the electron transfer chain in the mitochondria.

Our data correlate with previous results that showed that pretreatment with $CuSO_4$ prevented inhibition of mitochondrial complexes I, II, IV, V and Cu/Zn-superoxide dismutase induced by 1-methyl-4-phenylpiridine (MPP^+) in the rat striatum [24]. In this neurodegeneration model, $CuSO_4$ also reduced the MPP^+-induced increase in the enzymatic activity levels of caspases 8, 9 and 3, decreased apoptotic cell damage [25], and prevented the hypokinetic state in MPP^+-treated mice [26]. In mice, a copper-chelator led to reduced activity of complex IV in neurons and dropped activity of the anti-oxidant system in the brain tissue [27, 28]. Based on the above data, we can assume that the protective effect of copper ions in inhibiting of electron transport chain complexes may be associated with a direct effect on copper-dependent proteins or an indirect effect on apoptotic pathway proteins.

Conclusion

We showed that Cu^{2+} protected neurons against the toxicity induced by LPS, an inflammation inductor, or NaN_3, a cytochrome c oxidaseinhibitor. This protection is likely to be associated rather with Cu^{2+} interaction with Complex IV of the electron transfer chain in the mitochondria than with inhibition of NO production. Effects of Cu^{2+} on apoptosis pathway proteins also cannot be ruled out.

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Вклад авторов. Все авторы внесли существенный вклад в разработку концепции, проведение исследования и подготовку статьи, прочли и одобрили финальную версию перед публикацией.

Assessment of Mitochondrial Gene Activity in Dopaminergic Neuron Cultures Derived from Induced Pluripotent Stem Cells Obtained from Parkinson's Disease Patients

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Abstract

Introduction. *Induced pluripotent stem cells (iPSCs) culturing allows modelling of neurodegenerative diseases in vitro and discovering its early biomarkers.*
Our ***objective*** *was to evaluate the activity of genes involved in mitochondrial dynamics and functions in genetic forms of Parkinson's disease (PD) using cultures of dopaminergic neurons derived from iPSCs.*
Materials and methods. *Dopaminergic neuron cultures were derived by reprogramming of the cells obtained from PD patients with SNCA and LRRK2 gene mutations, as well as from a healthy donor for control. Expression levels of 112 genes regulating mitochondrial structure, dynamics, and functions were assessed by multiplex gene expression profiling using NanoString nCounter custom mitochondrial gene expression panel.*
Results. *When comparing the characteristics of the neurons from patients with genetic forms of PD to those of the control, we observed variations in the gene activity associated with the mitochondrial respiratory chain, the tricarboxylic acid cycle enzyme activities, biosynthesis of amino acids, oxidation of fatty acids, steroid metabolism, calcium homeostasis, and free radical quenching. Several genes in the cell cultures with SNCA and LRRK2 gene mutations exhibited differential expression. Moreover, these genes regulate mitophagy, mitochondrial DNA synthesis, redox reactions, cellular detoxification, apoptosis, as well as metabolism of proteins and nucleotides.*
Conclusions. *The changes in gene network expression found in this pilot study confirm the role of disrupted mitochondrial homeostasis in the molecular pathogenesis of PD. These findings may contribute to the development of biomarkers and to the search for new therapeutic targets for the treatment of SNCA- and LRRK2-associated forms of the disease.*

Keywords: *Parkinson's disease; SNCA; LRRK2; induced pluripotent stem cells; dopaminergic neurons; transcriptomics; mitochondria*

Ethics approval. Authors confirm compliance with institutional and national standards for the use of laboratory animals in accordance with «Consensus Author Guidelines for Animal Use» (IAVES, 23 July 2010). The research protocol was approved by the Local Ethics Committee of the Research Center of Neurology (protocol No. 5-5/22, June 1, 2022).

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Оценка активности митохондриальных генов в культурах дофаминергических нейронов, полученных из индуцированных плюрипотентных стволовых клеток от пациентов с болезнью Паркинсона

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Аннотация

Введение. *Технологии культивирования индуцированных плюрипотентных стволовых клеток (ИПСК) предоставляют возможность для моделирования нейродегенеративных заболеваний in vitro и поиска их ранних биомаркеров.*
Цель *исследования — оценить активность генов, вовлечённых в функционирование митохондрий, на культурах дофаминергических нейронов — производных ИПСК — при генетических формах болезни Паркинсона (БП).*

Материалы и методы. *Культуры дофаминергических нейронов были получены путём клеточного репрограммирования от пациентов с БП, являющихся носителями мутаций в генах SNCA и LRRK2, а также от здорового донора (контроль). С помощью технологии мультиплексного профилирования генной экспрессии на платформе «NanoString» оценивали экспрессию 112 генов, участвующих в структурно-функциональной организации митохондрий и собранных в специальную «митохондриальную» панель.*

Результаты. *При сравнении характеристик нейронов, полученных от пациентов с генетическими формами БП и в контроле, выявлены различия в активности генов, продукты которых связаны с работой митохондриального дыхательного комплекса, ферментами цикла трикарбоновых кислот, биосинтезом аминокислот, окислением жирных кислот, метаболизмом стероидов, гомеостазом кальция в клетке, утилизацией свободных радикалов. Ряд генов показал также дифференцированную экспрессию в культурах с мутациями SNCA и LRRK2; в дополнение к указанным выше функциям данные гены контролируют митофагию, синтез митохондриальной ДНК, окислительные реакции, процессы детоксикации клетки и апоптоз, метаболизм белков и нуклеотидов.*

Заключение. *Выявленные в настоящем пилотном исследовании изменения экспрессии генных сетей подтверждают роль нарушений митохондриального гомеостаза в молекулярном патогенезе БП и могут способствовать разработке биомаркеров и поиску новых терапевтических мишеней при SNCA- и LRRK2-ассоциированных формах заболевания.*

Ключевые слова: *болезнь Паркинсона; SNCA; LRRK2; индуцированные плюрипотентные стволовые клетки; дофаминергические нейроны; транскриптомика; митохондрии*

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Introduction

Parkinson's disease (PD) is a common age-related neurodegenerative disorder that primarily affects the dopaminergic neurons in the substantia nigra pars compacta (SNpc), resulting in a complex combination of motor and non-motor symptoms. By 2040, the number of people with PD is expected to reach 12.9 million [1]. All current PD treatments are symptomatic and do not stop the disease from progressing. The first motor symptoms occur when the dopaminergic neurons in the SNpc have already degenerated by about 60 %, which is why therapy is initiated too late [2]. Current technologies allow to culture induced pluripotent stem cells (iPSCs) obtained from the PD patients, thus providing new opportunities to study the pathogenesis of neurodegenerative disorders. The *in vitro* PD models and neurons derived from the iPSCs of PD patients with mutations in PD-causing genes appeared to be highly informative for identifying molecular drivers of the neurodegenerative process [3]. It is important to mention that iPSC-based models would help to identify the earliest morphological and functional changes in neurons and to detect the developing disease at its earliest presymptomatic stages.

Rapid advances in molecular technologies allowing efficient and powerful qualitative and quantitative assessment of various genetic characteristics have brought the studies in the field of disease progression markers to a new level. These include the NanoString nCounter technology developed by NanoString Technologies, which enables targeted multiplex analysis of hundreds of genes in a single run [4, 5]. The advantages of this technology over traditional gene expression analysis are walk-away automation of the workflow, robust performance, and reproducibility of the results. The sensitivity of the method is comparable to the one of a real-time PCR [6]. The method is based on molecular barcoding, in which the studied targets are tagged with target-specific color-coded probe pairs that enable further detection of the captured targets [7]. As the preliminary steps of reverse transcription and amplification often leading to biased data are excluded from the workflow [8], this method demonstrates high level of accuracy and sensitivity with low concentrations and small volumes of the source material [4, 5].

Current studies of the mechanisms involved in the development of PD focus primarily on mitochondrial dynamics [9, 10]. In our research we used bar-coding multiplex gene expression profiling on the NanoString platform to assess the activity of genes involved in mitochondrial dynamics in the cultures of dopaminergic neurons derived from iPSCs of the patients with genetic forms of PD.

Materials and methods

Cell line culturing

Skin biopsy specimen were obtained from two patients with known genetic forms of PD and one healthy donor. One of the PD patients had a heterozygous duplication of exons 2–7 of the *SNCA* gene and the second had the heterozygous *G2019S* point mutation in the *LRRK2* gene. All patients were familiarized with the conditions of the study and signed an informed consent form. The study was approved by the local ethics committee of the Research Center of Neurology (protocol No. 11/12 dated 12 September 2012).

The cells from the primary homogeneous dermal fibroblast culture were reprogrammed into iPSCs. To reprogram the fibroblasts, we used Sendai virus because its reprogramming factors and DNA do not integrate into the genome of the cells studied. All the iPSC lines were cultured in the mTeSR medium (STEMCELL Technologies) on Matrigel-coated substrates. Fibroblast reprogramming and iPSC differentiation into neural progenitor cells and further into neuronal cell cultures enriched by dopaminergic neurons were performed as previously described [11].

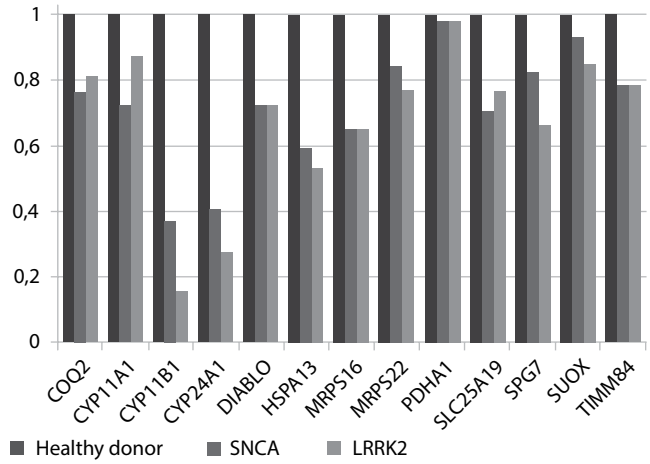
RNA isolation from the neuronal cell culture

Total RNA from the mature neuronal lines of the PD patients and the healthy donor was isolated using Total RNA purification kit (Norgen) according to the manufacturer's instructions. The RNA quantification was made using a Nanodrop 2000 spectrophotometer (ThermoScientific). The RNA isolate was used immediately or stored at –80ºC until used in the experiments.

Gene expression analysis

Gene expression was analyzed using NanoString technology (NanoString Technologies). The analysis used the custom gene expression panel containing 12 gene networks associated with dynamics and functions of mitochondria. The panel includes 112 genes, which were selected based on existing scientific data on their involvement in the regulation of mitochondrial structure and dynamics. The panel also includes 5 housekeeping genes as the controls. After hybridization of total RNA (100 ng) with the target-specific fluorescent tags, the samples were loaded into the prep station of the nCounter Analysis System (NanoString Technologies) for further digital analysis according to the manufacturer's protocols.

The data obtained were analyzed using nSolver v. 4.0 software. Source data were normalized using the housekeeping control genes included in the panel: β-actin (NM_001101.2), GAPDH (NM_002046.3), HPRT1 (NM_000194.1), RPL19 (NM_000981.3), and β-tubulin (NM_178014.2). The data obtained with the nCounter system are expressed in the units reflecting concentration of target RNA molecules in the sample.



Decreased expression of some genes associated with mitochondrial dynamics and functions in neurons derived from patients with genetic form of PD.

Results

For the iPSCs obtained from the PD patients and the healthy donor all necessary tests required by international standards were performed, namely the assessment of pluripotency marker expression, pluripotent cell gene expression, and karyotyping to confirm the normal karyotype of the cells and their ability to form embryoid bodies and derivatives of the three germ layers. The differentiation of the iPSCs from the PD patients and the healthy donor into neuronal progenitor cells was initiated simultaneously. The selection of iPSC lines was based on the results of the tests performed. The iPSC lines that showed a tendency towards preferential formation of neural derivatives in the spontaneous *in vitro* differentiation assay, were used first. Terminal differentiation into dopaminergic neurons was performed in two steps according to the previously used protocol [12].

Further, changes in mitochondrial gene expression profiles in three neuronal cell cultures derived from iPSCs were analyzed using the NanoString platform. We assessed expression levels of 112 genes from the custom NanoString Human mitochondrial panel. Comparative analysis revealed unidirectional changes (decreases) in the expression levels of 13 genes in the cell cultures from both patients with genetic forms of PD compared with those in the control neuron cell culture (see the Figure). In the known genetic forms of PD, a decrease in expression appeared to be typical for the genes associated with oxidative phosphorylation, the tricarboxylic acid cycle, amino acid biosynthesis, fatty acid oxidation, steroid metabolism, calcium homeostasis, and free radical quenching [13–15].

Table 1. Gene expression changes in the neurons with the *LRRK2* gene mutation

Metabolic pathway	Gene	Gene expression level
Mitochondrial respiratory chain	*SDHA*	Increased
	CYCS, ATP5E, ATPAF2, NDUFA1, NDUFB9, NDUFS4	Decreased
Transmembrane transport of substrates	*SLC25A12, SLC25A13, SLC25A FXN, TMLHE*	Increased
Tricarboxylic acid cycle	*FH*	Increased
Metabolism of the proteins, nucleotides, and vitamins	*AMT, PCCA, TMLH*	Increased
	GATM, GCDH, PCCB, HADHA	Decreased
Heat shock proteins	*HSPA1A, HSPA4L, HSPA6, HSPB1*	Decreased
Mitophagy	*PINK1*	Decreased
Protein translation	*TSFM*	Decreased

Table 2. Gene expression changes in the neurons with the SNCA gene mutation

Metabolic pathway	Gene	Gene expression level
Mitochondrial respiratory chain	*COX15, COX6B1, CYP11B2, CYP27A1, ETFA, MT-ATP6, MT-ATP8, MT-CO1, MT-CO2, MT-CO3, MT-CYB, MT-ND1, MT-ND2, MT-ND3, MT-ND4, MT-ND4L, MT-ND5, MT-ND6, NDUFA10, NDUFA11, NDUFB3, NDUFS2, NDUFS3, NDUFS6, NDUFV1, SDHB, SDHC, SDHD*	Increased
	UQCRB, COX10	Decreased
Transmembrane transport of substrates	*ABCB6, CPT1A, SLC25A20, SLC25A4, TIMM44*	Increased
	SLC25A15, SLC25A22, SLC9A6	Decreased
Tricarboxylic acid cycle	*SUCLA2, PDHB, PDHX*	Increased
Mitophagy	*GSR*	Increased
	HIF-1α, Mfn2, OPA1	Decreased
Amino acid metabolism	*HADHB*	Increased
	ALDH18A1, NDUFV2, SARDH	Decreased
Heat shock proteins	*HSPA9*	Increased
	HSPA14	Decreased
Replication and repair of mitochondrial DNA	*DGUOK, POLG, C10orf2*	Decreased
Protein translation	*TUFM, MRPL3*	Decreased
Gem synthesis	*PPOX*	Decreased

Decreased expression of some genes associated with mitochondrial dynamics and functions in neurons derived from patients with genetic form of PD.

Several genes showed a differential expression in the neurons cultured from the PD patient cells with mutations in the *LRRK2* and *SNCA* genes. In the neurons with the *LRRK2* gene mutation 10 genes showed an increase in expression and 16 genes showed a decrease in expression compared to the control neuron cell culture and the culture of neurons with the *SNCA* gene mutation (Table 1). The products of these differentially expressed genes are involved in the mitochondrial respiratory chain, the tricarboxylic acid cycle, mitophagy, protein processing and metabolism of the proteins, nucleotides and vitamins in a cell, transmembrane transport of iron and other substrates [16, 17].

In the neurons derived from the cells with the *SNCA* gene mutation 44 genes showed increased expression levels and 21 genes showed decreased expression levels compared to the control and to the neurons with the *LRRK2* gene mutation (Table 2). Increased expression was observed in genes involved in oxidative phosphorylation, mitophagy, replication and repair of mitochondrial DNA, the tricarboxylic acid cycle, protein processing, lipid and protein metabolism, redox control, apoptosis, and protection against neurotoxicity. The detected genes with decreased expression are mainly involved into protein sorting and accumulation, and protein and nucleotide metabolism [18–23].

Discussion

Mitochondria play a key role in regulating cellular bioenergetics. Involved in numerous signaling pathways, they contribute to the development, plasticity, and differentiation of neurons, including the activation of cell apoptosis [24].

In our pilot study, we assessed the expression levels of more than 100 genes associated with mitochondrial dynamics and functions using the NanoString platform. Comparative analysis of the characteristics of the neurons obtained from the patients with genetic forms of PD and a healthy donor, allowed us to identify the differences in the expression of genes regulating oxidative phosphorylation activity, the tricarboxylic acid cycle, amino acid biosynthesis, fatty acid oxidation, steroid metabolism, calcium homeostasis, and free radical quenching. Several genes showed a differential expression in the cell cultures with the *SNCA* and *LRRK2* gene mutations. These genes, in addition to the functions mentioned above, regulate mitophagy, mitochondrial DNA synthesis, redox reactions, cellular detoxification and apoptosis, and lipid and protein metabolism.

The identified changes in gene network expression confirm the role of disrupted mitochondrial homeostasis in the molecular pathogenesis of PD. These findings may contribute to the development of new biomarkers and to the search for new therapeutic targets for the treatment of *SNCA*- and *LRRK2*-associated forms of the disease.

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Poststroke Asthenic Disorder

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Abstract

Asthenic disorders are seen in approximately half of poststroke patients. The mechanisms underlying poststroke asthenia (PSA) are related to brain connectome damage, as well as neuroinflammatory and neuroendocrine mechanisms. PSA is associated with a lack of energy, lassitude, and fatigue that do not improve after rest or sleep; it is differentiated from depression, apathy, and daytime sleepiness. Risk factors for PSA include female gender, anxiety and depressive disorders, severe neurological deficit, sleep disorders, diabetes etc. Treatment of PSA includes cognitive behavioral therapy graded physical activity, and pharmacotherapy.

Keywords: *stroke; asthenia; fatigue; tiredness; depression*

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Астеническое расстройство после инсульта

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Аннотация

Астенические расстройства отмечаются примерно у половины пациентов после инсульта. Механизмы развития постинсультной астении (ПИА) связаны с поражением коннектома головного мозга, нейровоспалительными и нейроэндокринными механизмами. Для ПИА характерны нехватка энергии, вялость, быстрая утомляемость, которые не уменьшаются после отдыха или сна; её дифференцируют с депрессией, апатией и дневной сонливостью. Факторами риска по развитию ПИА являются женский пол, тревожно-депрессивные расстройства, выраженный неврологический дефицит, нарушения сна, сахарный диабет и др. В лечении ПИА используются методы когнитивно-поведенческой терапии, дозированные физические нагрузки, фармакотерапия.

Ключевые слова: *инсульт; астения; усталость; утомляемость; депрессия*

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Asthenic disorder is a condition manifested by increased tiredness with very unstable mood, reduced self-control, impatience, restlessness, disturbed sleep, loss of the ability for prolonged mental and physical effort, as well as intolerance to loud sounds, bright light, and strong odors [1]. Asthenic disorder is a major compo-

nent of neurasthenia (i.e. a form of neurotic disorder) and can be observed in patients with medical conditions or central nervous system (CNS) damage [1, 2]. The prevalence of asthenic disorder in the general population varies from 1.5% to 15% [1]. Asthenia is observed in half of patients with Parkinson's disease [3]; in patients with multiple sclerosis its prevalence can be up to 78% [4].

In Western scientific literature, authors traditionally use the more general term "fatigue", which reflects only the major manifestation of asthenia. This term implies pathological fatigue, which, unlike physiological fatigue, does not depend on previous physical activity and does not decrease after rest or sleep [5].

Asthenia (pathological fatigue) is a significant challenge to poststroke patients. According to experts, this phenomenon is one of the key areas for research when studying stroke sequelae [6]. Given that the terms "asthenia" and "pathological fatigue" are essentially the same, we will use the term "poststroke asthenia" (PSA) in this article. Currently there is no commonly accepted definition of PSA. Some authors define it as a state of subjective feeling of fatigue and exhaustion that develops regardless of previous stress and does not decrease after rest or sleep [7].

Epidemiology

PSA is observed in many patients; according to several authors, its prevalence can be up to 90% [7]. T. B. Cumming et al. conducted a systematic review of 22 studies that used the Fatigue Severity Scale (FSS) to assess PSA. The average prevalence of PSA was 50% (95% confidence interval [CI] 43–57%). In comparative analysis, fatigue prevalence was found to be lower in studies conducted in Asian countries (35%; 95% CI 20–50%) [8]. A meta-analysis conducted by I. Alghamdi et al. in 2021 included 35 studies (6851 patients) and yielded comparable results; PSA prevalence was 48% (95% CI 42–53%) and 48% (95% CI 43–53%) using FSS and Multi-dimensional Fatigue Inventory (MFI), respectively [9].

The prevalence of PSA remains high both in the acute period and several years after stroke. According to A. Pedersen et al., in 7 years post-stroke, up to 80% of the patients reported PSA symptoms [10].

Pathophysiological mechanisms

Currently, the mechanisms underlying asthenia in patients with neurological disorders are considered from the perspective of the set point theory. According to this theory, the body can function in an optimal way only while being in a state of balance between various multidirectional physiological processes. This is called a set point. Disturbed set point leads to asthenia. This is caused by various mechanisms [2]. They can be classified into neuroimmune, neuroendocrine, neurochemical, neurophysiological, etc. [11].

The pathogenesis of PSA is currently not well understood. A single experimental PSA study was conducted by A. Kunze et al. [12]. Its results showed significant differences in behavioral correlates of fatigue and depression in different rat strains for up to 50 days after experimental stroke. In particular, Sprague-Dawley (SD) and Wistar rats increased spontaneous activity during the light cycle, when the rodents are not active, and decreased spontaneous activity during the dark cycle, when they should be active, which is an equivalent of fatigue. Lewis rats had high spontaneous activity during the dark cycle but increased duration of immobilization in the forced swim test, which was consistent with the phenomenon of learned helplessness and considered to be an equivalent of depressive disorder in this species. Lewis rats also had significantly increased serum levels of interleukin-10 *vs*. SD and Wistar rats. The authors concluded that these changes were related to inter-strain differences in the development of the immune response, and PSA and depression were caused by different neuroimmune mechanisms related to poststroke aseptic inflammation [12]. Inflammation is associated with increased blood levels of cytokines and leads to neurochemical disturbances in the CNS due to inhibition of indoleamine 2,3-dioxygenase and switching of tryptophan metabolism from serotonin to kynurenine, a neurotoxic compound [13].

Neuroimmune mechanisms are likely to play the most significant role in the development of so called early-onset fatigue, which is seen during the first year after stroke [14]. W. de Doncker et al. provided information that cytokine and kynurenine blood levels were higher and tryptophan index (ratio of tryptophan to competing amino acids) was lower in patients with fatigue 12 months post-stroke. At 18 months post-stroke, this pattern was not seen [15].

Subsequently, the neuroimmunological theory of PSA was confirmed in molecular genetic studies, which showed that patients carrying C allele of interleukin-1 receptor antagonist gene were more prone to develop poststroke asthenia. On the other hand, TLR4 allele carrier status, which has anti-inflammatory properties, reduced the risk of PSA [16].

The neuroendocrine theory explains the development of PSA by the insufficiency of the endocrine glands and by metabolic disorders. According to different authors, the prevalence of poststroke pituitary dysfunction can be up to 82%. Somatotropic hormone insufficiency is the most common. Somatotropic hormone is produced in the lateral parts of the anterior pituitary, which

is most susceptible to damage during stroke. Data on the relationship between pituitary insufficiency and PSA are inconclusive. The role of neuroendocrine dysfunction in PSA is being investigated in the PIT-FAST study [17].

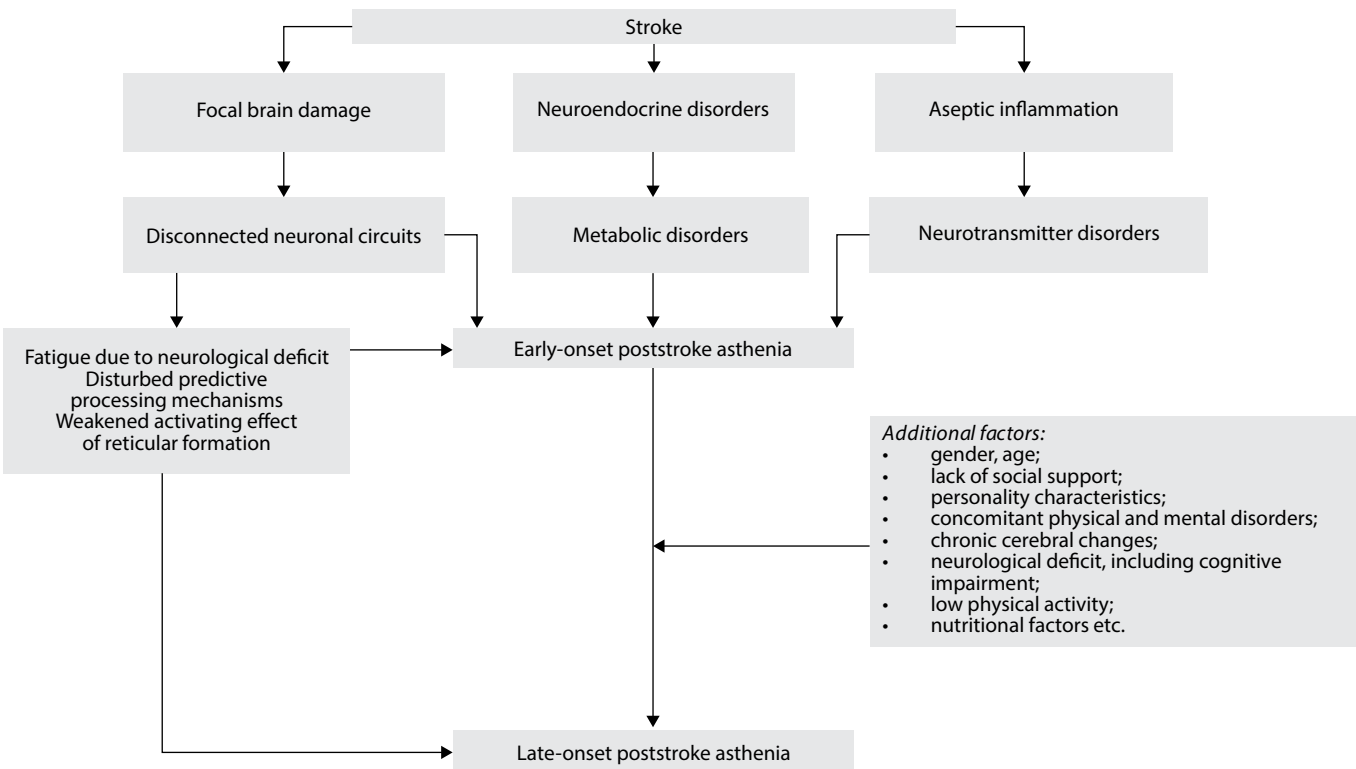
A study using brain magnetic resonance imaging (MRI) with diffusion tensor imaging (DTI) and resting-state functional mapping showed that disruptions in structural and functional connectivity play an important role in the development of PSA after middle cerebral artery stroke. The most important changes include connectivity disruptions in the ipsilesional rostral middle frontal cortex, with greater structural disconnection correlating with more severe asthenia [18].

Recently, several neurobiological theories explaining PSA development have been proposed. Motor activity is normally accompanied by the perception of effort required for muscle contraction. Perceived effort results from the integration of afferent input from muscles and sensory prediction. The sensory attenuation model explains the development of asthenia by increased perception of effort due to disturbed gate control mechanism of motor sensations [15].

The metacognitive dyshomeostasis model is related to predictive coding theory (predictive processing). According to this theory, the brain constantly creates and updates its internal model of the environment. It generates internal afferent input, which is then compared with the actual afferent input. If they do not match with each other, prediction errors are formed, which are then used to update the internal model. Many predicting errors make the brain to pay more attention to internal input, which in turn reduces the patients' self-confidence (i.e. self-efficacy), and this may underlie the development of asthenic disorders.

The inhibitory sensitization model explains the development of asthenia by the fact that excessive excitation of the activating systems of the brain increases the sensitivity of the inhibitory systems of the CNS. This leads to constant signaling from the brain about the need for rest, which makes the patient feel exhaustion and fatigue.

Considering the data presented, we can assume that different mechanisms may underlie early-onset and late-onset PSA. Biological mechanisms (e.g. neuroimmune, neuroendocrine, etc.) play a more significant role in the development of early-onset asthenia, while additional factors (e.g. age, comorbidity, etc.) lead to the chronic process and late-onset asthenia (Figure). The said theories can explain PSA only partially, so its mechanisms are still being investigated [15].



PSA pathogenesis model.

Factors associated with PSA

Group of factors	Factors
Demographic and socioeconomic	Female gender, low social support
Psychological/psychophysiological	Depression, anxiety, cognitive impairment, external locus of control, coping strategy (avoidance, confrontation), sleep disorders
Neurological	Severe neurological deficit (assessed by NIHSS, mRs), hemorrhagic stroke [20]
Laboratory	Decreased thyroid-stimulating hormone [22], increased uric acid [23], cytokines, neutrophil-lymphocyte ratio, decreased prognostic nutritional index [24], increased C-reactive protein [25]
Physical	Arterial hypertension and hypotension, type 2 diabetes, musculoskeletal pain, cardiac arrhythmia, obesity [26]
Neuroimaging	Strokes involving the thalamus, basal ganglia, or infratentorial structures; leukoaraiosis
Pharmacological	Statins, antidepressants, muscle relaxants, polypharmacy

Note. NIHSS, National Institutes of Health Stroke Scale; mRs, modified Rankin scale.

Data on the association between stroke lesion localization and PSA are of special interest. Damage to the brain stem and basal ganglia is thought to lead to asthenia due to damage to the ascending activating reticular formation and changes in the volitional sphere, respectively. However, a meta-analysis conducted by J. Shu et al. did not confirm any significant association between stroke location and PSA [20]. The stroke lesion itself and, therefore, partial disconnection of the brain connectome might be sufficient to significantly increase the likelihood of developing asthenic disorder. This hypothesis was confirmed by C. Winward et al., who showed that the prevalence of fatigue was higher after minor stroke than after transient ischemic attacks. In other words, transient symptoms were observed in both groups clinically but, in patients with stroke, an acute ischemia lesion was detected, which probably caused a higher prevalence of fatigue in this group [21].

Some factors may play a dual role in the development of PSA. For instance, obese patients were less likely to develop asthenia in the acute stage of stroke; however, they were more prone to fatigue 6 months post-stroke [26].

A systematic review of studies investigating the association between PSA and cognitive impairment gave inconclusive results. Four studies found significant correlations between PSA and memory, attention, speed of information processing, and reading speed (r from −0.36 to 0.46), whereas seven studies did not [27]. The role of speech disorders in PSA has not been established completely due to the fact that aphasia is a common exclusion criterion in many studies [28].

Low physical activity before and after stroke may predispose the patient to the development of asthenia. A systematic review by F. Duncan et al. did not show any evident association between fitness and PSA [29]; however, D. Tai et al. in their meta-analysis showed that physical exercise improved pathological fatigue in post-stroke patients [30].

It is especially important to define modifiable PSA risk factors, which may include chronic pain, increased anxiety, polypharmacy, uncontrolled medical conditions, and low level of physical activity. Managing these factors can be considered an approach to the prevention and treatment of PSA.

Socioeconomic factors play an important role in the development of stroke and its complications [31]. Low level of social support was shown to increase risk of PSA [7].

Considering the high prevalence of asthenia after COVID-19, we can assume that the risk of PSA development after COVID-19-related stroke might be increased [32]. However, this has not been studied yet.

Assessment of poststroke asthenia

Several scales are used to assess PSA. The most popular scale is the Fatigue Severity Scale (FSS), which consists of 9 items, with each item scored from 1 to 7. It allows differentiating PSA from depression; however, its sensitivity in evaluating pathological fatigue over time may be inadequate. The Fatigue Impact Scale (FIS) consists of 40 items, with each item scored from 0 to 4. The disadvantage of this psychometric tool is a relatively large number of items. The Modified Fatigue Impact Scale (mFIS) does not have this drawback and consists of 21 items. Its disadvantages include the fact that it is mainly aimed at assessing the impact of pathological fatigue on the patient's daily living. Other scales include Fatigue Assessment Scale and Vitality Subscale of the SF-36 scale. The simplest tool to assess PSA is a fatigue visual analogue scale. However, its validity is significantly lower than that of the above-mentioned scales [7]. When using the scales, the patient is diagnosed with PSA if their score is higher than a certain value. The scores can be also used to assess the severity of fatigue.

J. Lynch et al. proposed a case definition for pathological fatigue after stroke in inpatients and community patients. It also allows differentiating PSA from daytime sleepiness [5].

PSA case definition for community patients. The patient has felt fatigue, a lack of energy, and increased need to rest every day or nearly every day for at least 2 weeks in the past month. This fatigue has led to difficulty taking part in everyday activities.

PSA case definition for inpatients. Since their stroke, the patient has experienced fatigue, a lack of energy, or an increased need to rest every day or nearly every day. This fatigue has led to difficulty taking part in everyday activities (for inpatients this may include therapy and may include the need to terminate an activity early because of fatigue). If the patient's condition conforms to this definition, they can be diagnosed with PSA.

A comparative analysis of the above definitions showed that they assess more the impact of PSA on daily activities, while many scales rather reflect PSA symptoms. A disadvantage of the case definition is its inability to assess PSA severity and characteristics. PSA can be diagnosed using either the scales or case definition; however, more sensitive and specific PSA assessment tools are still being developed.

Differential diagnosis

Differential diagnosis of PSA includes clinically similar disorders such as apathy, depression, and hypersomnia. All of these phenomena are characterized by decreased motor activity, lassitude, impaired social functioning, and negative impact on stroke outcome [33].

Apathy is a common poststroke phenomenon, which is characterized by decreased motivation, limited goal-directed behavior, including its emotional component, and limited goal-directed cognitive activity. Prevalence of poststroke apathy is over 50% [34]. Apathy after stroke is associated with older age, damage to the frontal-subcortical region of the brain, and decreased cognitive function. Studies of poststroke apathy and asthenia showed that these two phenomena are not related to each other [35, 36].

Asthenia can be seen in patients with depression. However, depression is more complex phenomenon than asthenia; clinical presentation of depression includes 3 components: emotional disturbances (dysthymia, anhedonia, feeling of hopelessness), negative cognitions (self-blame and self-effacement ideas) and behavioral disorders (decreased appetite, slowness, asthenia) [37]. E. Douven et al. showed that these two phenomena are interconnected both in the acute and recovery period of stroke. However, several studies demonstrated fundamental differences between the two phenomena. In particular, only half of patients with PSA have symptoms of depression, and antidepressants (e.g. fluoxetine) can reduce the severity of depression but not asthenia; moreover, some antidepressants such as tricyclics can exacerbate asthenia [36].

Hypersomnia (daytime sleepiness) can be similar to asthenia but represents an increased likelihood of falling asleep in certain situations. Unlike hypersomnia, PSA does not improve after sleep. However, it is of note that both phenomena are associated, to one degree or another, with poststroke sleep disorders [7].

Besides pathological fatigue, some authors distinguish pathological fatigablility. The latter is a responce to increased exertion when performing certain actions due to a neurological deficit. For example, one patient with dysphasia and another patient with leg paresis would feel very tired after a normal conversation or walking, respectively. However, pathological fatigability usually is seen in combination with pathological fatigue, which is observed regardless of the exertion. Therefore, classifying pathological fatigability as a separate phenomenon is doubtful, and it is usually considered only as one of the mechanisms underlying poststroke asthenic syndrome [38].

Diagnosis of PSA usually does not require any additional investigation methods; however, if patients experience concominant symptoms (e.g. edema, bradycardia, pale skin, brittle nails, etc.) or an unusual course of asthenia, when it worsens over time, a medical condition (e.g. anemia, hypothyroidism, chronic inflammatory disease, diabetes, sleep apnea, etc.) can be suspected [7]. In such cases, patients should be screened for these conditions.

Treatment

There is an unmet medical need for patients with PSA [39]. Potentially effective therapies for PSA include psychostimulants. In particular, the randomized controlled trial (RCT) MIDAS demonstrated the efficacy of modafinil 200 mg/day [40]. However, in another RCT modafinil did not have any significant effect on MFI-20 score but improved overall score of the FSS scale and vitality subscore of the SF-36 scale. An analysis conducted in Australia showed economic benefits of modafinil for patients with PSA of working age [41]. Few open studies conducted by Russian authors demonstrated the effect of medications such as sulbutiamine, phenylpiracetam, and idebenone in the treatment of PSA [7, 39].

Literature data reported a positive effect of vitamin D supplementation in poststroke patients with asthenia and vitamin D deficiency [42]. Studies of Chinese authors showed positive effects of therapies popular in the Oriental medicine such as herbal remedies with *Astragalus membranaceus* and electroacupuncture [39].

Transcranial direct current stimulation is a non-invasive neuromodulation method based on treatment of the cerebral cortex by weak electric field. A RCT conducted by X.L. Dong et al. showed that a 4-week course of transcranial direct current stimulation significantly improved PSA vs. placebo. However, there were no inter-group differences at Month 8 [43].

Non-medication interventions for PSA also include psychoeducation, cognitive behavioral therapy, multicomponent programs, mindfulness-based stress reduction therapies [44], and complex rehabilitation [45]. A systematic review conducted by D. Tai et al. showed that exercise training reduced PSA [30]. According to the COGART RCT, the highest efficacy was demonstrated by a combination of cognitive behavioral therapy with graded physical exercise [46].

C.H. Teng et al. conducted a systematic review of patients' strategies for adaptation to PSA [47]. An important role in adaptation to PSA is played by family members, employers, and colleagues; however, the authors could not find out how close ones can contribute to this process. In the opinion of stroke survivors, PSA should be addressed by training them and their close ones on key approaches to its management.

The adaptation process includes adaptation to asthenia itself, daily activities, and the patient's role in the society with the account of the limitations associated with the stroke. In poststroke patients, asthenia has a negative impact on all areas of activities such as physical, cognitive, mental, and social functions. Therefore, a multidisciplinary team, in collaboration with the patient and their caregivers, should develop a comprehensive strategy to better adapt the patient to PSA.

Impact on stroke survivors' life

PSA has a negative impact on various aspects of stroke survivors' life and impairs their recovery from poststroke neurological deficit [48-51]. In particular, PSA in the acute period of the first stroke is a predictor of low activity of daily living at discharge [51] and 1.5 years post-stroke [50], regardless of the presence of depression. Based on the data available, the authors concluded that treatment of PSA can be considered as an option to increase the effectiveness of rehabilitation measures; however, clinical studies are needed to confirm this assumption.

PSA reduces the patient's quality of life [52, 53], instrumental activities of daily living [54], increases the risk of suicidal behavior [55], and reduces the likelihood of returning to work after stroke even in patients without significant neurological deficit [56].

Conclusion

Up to half stroke survivors have asthenic disorder. The mechanisms underlying PSA are being investigated; they are likely to be related to brain connectome damage, as well as neuroinflammatory and neuroendocrine mechanisms. To diagnose PSA, the healthcare provider should ask the patient about their symptoms such as lack of energy, lassitude, and fatigue that do not decrease after rest or sleep; differential diagnosis of PSA should include depression, apathy, and daytime sleepiness. Factors predisposing patients to PSA include female gender, older age, anxiety and depressive disorders, severe neurological deficit, sleep disorders, diabetes, etc. The most effective treatment options for patients with PSA include cognitive behavioral therapy, graded physical activity, and, in more severe cases, psychostimulants.

Further studies are needed to clarify the mechanisms underlying PSA and, based on their results, treatment options with high efficacy should be developed for patients with PSA. When planning rehabilitation in stroke survivors, it is necessary to consider the high risk of developing PSA in this population.

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Neurobehavioral Testing as Cognitive Function Evaluation tool in Experimentally Induced Neurodegeneration in Mice

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Abstract

Neurodegeneration is a complex and multifactorial process presenting one of the major issues of fundamental science and clinical medicine due to its high prevalence, multiple nosological entities, and variations in pathogenesis. Translational research contributes to the study of neurodegenerative diseases, with modeling of such pathologies being an important part of this research. Behavioral testing in various animal models of neurodegenerative diseases allows to assess the model validity and reliability, as well as to investigate the potential efficacy of pharmacotherapy and other management approaches. In this overview we present test batteries that evaluate behavior, cognitive performance, and emotional states in animals with experimentally induced neurodegeneration.

Keywords: *neurodegeneration; memory; fear conditioning; conditioned freezing; neurogenesis*

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Нейроповеденческое тестирование как инструмент оценки когнитивных функций при экспериментальной нейродегенерации у мышей

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Аннотация

Нейродегенерация — это сложный и многофакторный процесс, являющийся одной из серьёзных проблем фундаментальной науки и клинической медицины ввиду распространённости, множества нозологических форм и вариаций патогенетических механизмов. Трансляционные исследования способствуют изучению нейродегенеративных заболеваний, а немаловажной частью данного процесса является моделирование патологий. Поведенческое тестирование животных с различными моделями нейродегенеративных заболеваний позволяет оценить степень достоверности моделирования, а также рассмотреть эффективность потенциальной лекарственной терапии и других типов коррекции. В данном обзоре представлена подборка батареи тестов, применяемых для оценки поведения, когнитивных функций, эмоционального статуса у животных с экспериментальной нейродегенерацией.

Ключевые слова: *нейродегенерация; память; кондиционирование страха; условно-рефлекторное замирание; нейрогенез*

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Introduction

Studying the pathogenesis of various neurological and neurodegenerative diseases alongside with ageing processes has been a critical focus of modern neurobiology for many years. The need to improve patients' life expectancy and quality of life drives the relevance of these studies. Thus, the modeling of neurodegeneration processes and the development of related therapies represent significant challenges of modern times.

Neurodegenerative diseases include several nosological entities characterized by neuronal degeneration, which may be chronic, as in Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis, Huntington's disease (HD), Lewy body dementia, and acute, as in cerebral infarction or central nervous system (CNS) trauma [1, 2]. The pathogenesis of this group of diseases is characterized by the gradual degeneration of neurons, reduced cerebral blood flow, and blood-brain barrier dysfunction, resulting in progressive behavioral and cognitive impairment. There is an urgent need for novel strategies in the recovery, management, and prevention of cognitive dysfunction. Therefore, comprehensive translational research *in vivo* is fundamental in the development of personalized therapy. Translational research allows to identify the morphological substrates and mechanisms underlying the pathogenesis of these diseases and their manifestations, and facilitates the translation of research data from preclinical studies to clinical applications.

When selecting a modeling method for a specific pathology, it is crucial to consider the validity, availability, and reproducibility of neurological symptoms and behavioral impairment that are pathognomonic of these diseases [3]. Neurobehavioral phenotyping in experimental animals allows the evaluation of various agents, therapeutic methods and approaches, as well as the associated risks, paving the way for translational approach in neurobiology [4].

Experimental mouse models of neurodegenerative diseases

Rodents have been the most extensively utilized models in experimental research, especially mice because of their genetic and physiological resemblances to humans [4–7]. According to B. Ellenbroek et al., there has been a shift in the proportion of neuroscience research using mice from about 20% in the 1970s and 1980s to around 50% in recent years [8].

Rats and mice belong to the Muridae family. Although they share many similarities, certain differences between these rodents are of critical importance for neuroscience research. Although the brains of rats and mice are anatomically identical, several significant functional dissimilarities have been identified that could impact animal behavior and research findings [8]. The larger size of the brain and spinal cord in rats offers several practical benefits for surgical procedures and targeting specific brain structures [8, 9]. Meanwhile, mice are better suited for optogenetic studies [10, 11] due to the easier penetration of light into the deeper structures of their smaller brain.

Over 95% of mouse genes have a sequence match in the human genome [12, 13], making murine models valuable surrogates for human disease studies, including the neurodegeneration research.

The lifespan of mice compared to humans is relatively short, with one human year being roughly equivalent to nine mice days [6]. To establish a precise correlation between the age of a mouse and a human, reference points for their lifespans and corresponding age ranges are used [14–16].

There are various ways to model neurodegeneration in rodents. The predominant method involves administering a neurotoxic agent directly into the rodent CNS, while the use of genetically modified animals is less common. For example, the intrahippocampal injection of amyloid-β is a traditional injection-induced model for AD [17–19]; HD models are based on quinolinic acid, PD models may be based on systemic injection of 6-hydroxydopamine (6-OHDA) [20, 21], intranigral injection of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) or its active metabolite 1-methyl-4-phenylpyridinium. Rotenone [22] and lactacystin [23] are also used to model this disease.

Genetic aspects of neurodegeneration can be studied using knockout and transgenic mouse lines. The advantages of transgenic animal models include a more comprehensive reflection of the pathogenesis and disease progression in several genetically determined diseases. For example, studies examining prion-like properties of tau-protein in relation to Alzheimer's disease employ hTau40/ΔK280 and hTau40/ΔK280/PP transgenic mice [24, 25], as well as transgenic mice with *ApoE4* [26, 27], *PSEN-1*, and *PSEN-2* [28, 29], APP-PS1 [30], APP23 [31, 32], and various other transgenic mouse lines [33, 34]. To study PD, α, β, γ-synuclein knockout mouse lines were generated [35]. BACHD, R6/2, R6/1, and YAC 128 mouse lines were created to explore HD [36–38].

Thus, the mouse models of neurodegenerative diseases enable more accurate and reproducible study results with more practical advantages over other rodent models.

Modern methods of phenotype and cognitive function assessment in mouse models of neurodegenerative diseases

The primary focus of a mouse model-based research of neurodegeneration is the assessment of animal be-

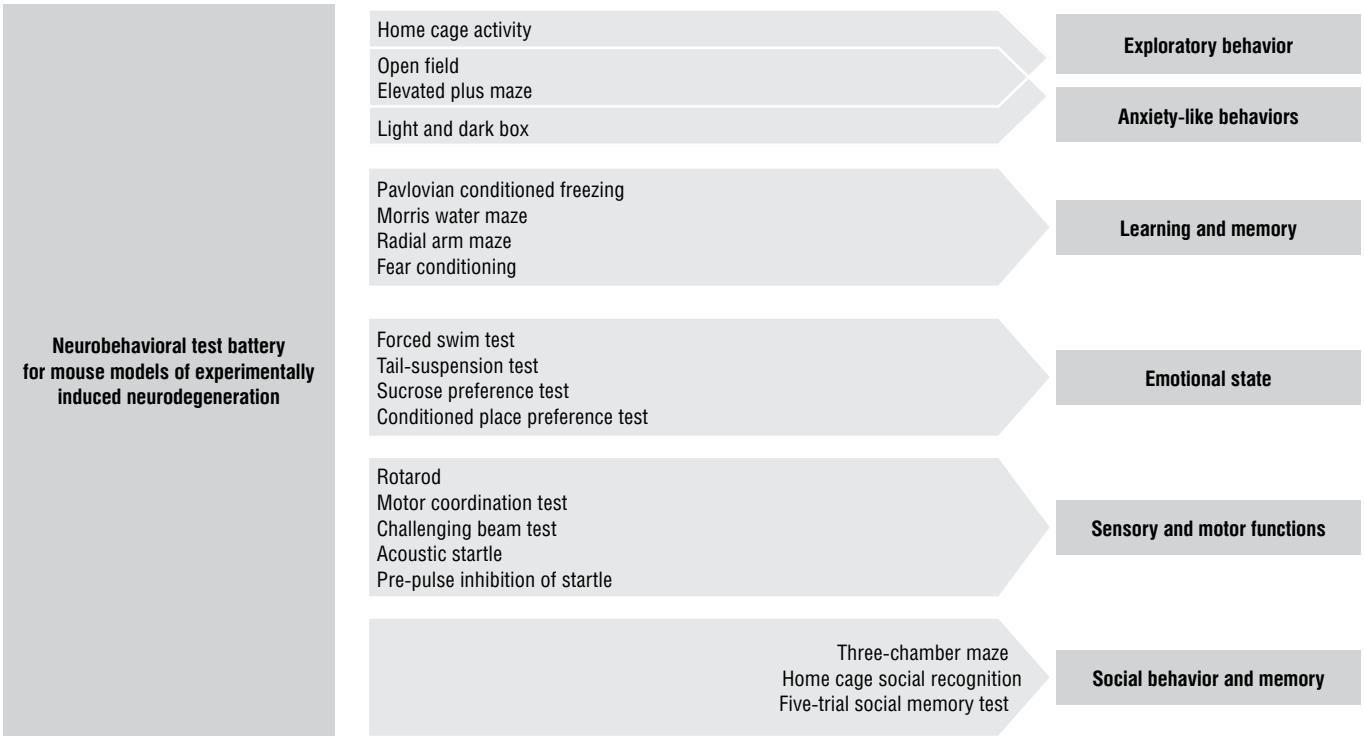

Fig. 1. A diagram of neurobehavioral testing in experimental mouse models of neurodegeneration.

havior and cognitive functions. Neuronal degradation manifests as altered behavior and memory, which can be evaluated through non-invasive methods to measure these neurodegenerative changes.

Neurobehavioral tests can be divided into several large groups based on behavioral parameter to be assessed (Fig. 1):
1) general assessment of anxiety or experimental psychiatric tests;
2) learning and memory tests;
3) assessment of emotional states;
4) sensory and motor tests;
5) exploratory behavior tests;
6) social interaction tests.

We will further provide detailed information regarding the most commonly used tests in mouse models of experimentally induced neurodegeneration.

General assessment of anxiety

In experimental modeling of neurodegeneration, it is crucial to determine whether the model matches the clinical phenotype of the specific disease. The traditional triad of primary behavioral assessment tests includes the open-field, the elevated plus maze, the light and dark box tests which can be a starting point for further research. All three tests may be used to evaluate the anxiety level, emotional behavior, and motor and exploratory activities of the animal [39]. They are simple to use with no need for expensive equipment. At the same time, they harmonize well with each other. Similar results obtained in these tests can be used to draw conclusions about the level of anxiety in the animal. At the same time, omitting one or choosing only one of the tests can result in baseline behavioral response biases.

Memory

One of the most severe clinical manifestations of neurodegeneration is the decline in cognitive functions, particularly memory [43, 44]. Given the pivotal role of memory in cognitive processes, comprehending the neural mechanisms of encoding, storing, consolidating, and reproducing information is imperative. To achieve this goal, we need to study individual neurons of the target brain structure and classify them into types based on gene expression levels, morphology, physiology, and their interactions with other neurons. It is typical to involve the entire network of neurons spread throughout the brain in performing cognitive tasks, including those associated with memory. At the same time, identifying the types of neurons localized in specific brain regions and the transmission of signals to underlying areas is essential [45, 46].

Nowadays, various subtypes of memory are known, including semantic memory, episodic memory, declarative memory, spatial memory, emotional con-

ditioning, procedural memory (skills and habits) [47]. Basically, memory is categorized into two types: declarative memory, also referred to as explicit memory, and non-declarative memory, also known as implicit memory. These types of memory are more distinguishable in humans than in animals [47, 48]. Both types of systems are independent, but they interact with each other to provide well-coordinated control over cognitive processes and behavior.

The declarative memory pertains to the recollection of personal experiences or events (episodic memory), or the factual knowledge of the world (semantic memory) [49, 50]. However, the information accumulated during our life is not limited by facts and episodes. There is also procedural non-declarative memory where information about our skills, habits, and behavior is stored making our recollections comprehensive [51].

Over the past decade, numerous studies have been conducted to identify the areas and systems of the brain responsible for various types of memory. Some studies were successful in understanding its mechanisms, but did not manage to identify memory engrams — subpopulations of neurons that bear specific memory traces. To pinpoint them, a combination of novel technologies were utilized: activation and regulation of immediate-early genes, transgenetics, optogenetics, pharmacogenetics, *in vivo* and *in vitro* cell physiology, and neurobehavioral testing [43, 52]. There are particular advances in research of classical conditioning effects regulated by the hippocampus and (or) the amigdala [48].

Studies of procedural non-declarative memory

Procedural memory, including the acquisition of a motor reflex to a sensory stimulus [45], has several specific traits. The acquisition of procedural memory traces involves two mechanisms: associative and non-associative learning. Non-associative learning refers to changes in the behavioral response to a certain stimulus over time, resulting from either habituation (a decline in response to a repeatedly presented stimulus) or sensitization (progressive amplification of a response to a repeatedly presented stimulus). Associative learning alters the behavior by establishing associations between events [53]. There are two types of associative learning: classical conditioning and instrumental conditioning [54].

Classical conditioning was first discovered and described by the Russian physiologist Ivan Pavlov at the end of the 19th century. Classical (Pavlovian) conditioning associates stimulus A causing a measurable response A with stimulus B, which normally does not cause response A. Stimulus A is an unconditioned stimulus, as a response to such a stimulus is elicited without any prior conditioning. Stimulus B is a conditioned stimulus since it requires conditioning to elicit a response. A learned response to a conditioned stimulus is a conditioned response [53, 54]. According to modern findings, this type of learning is predominantly regulated by the amigdala.

Instrumental conditioning was first discovered and studied at the beginning of the 20th century. In this learning mechanism, behavior or motor activity is associated with

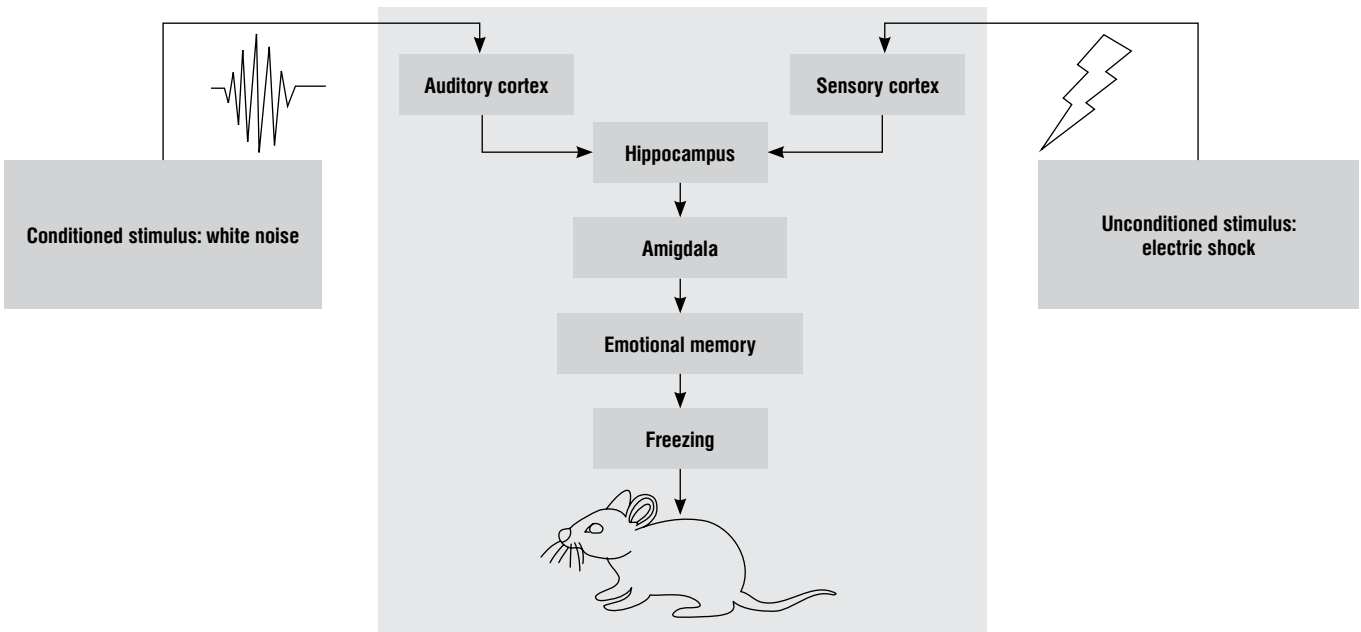

Fig. 2. The fear conditioning test to reveal the interaction between the auditory cortex, the hippocampus, and the amigdala nuclei in formation of the emotional memory.

a significant stimulus, for instance, edible reinforcement. Since motivation plays an important role in the instrumental conditioning (a well-fed animal will not be interested in performing any action for the sake of getting food), the physiology of instrumental conditioning is more intricate than that of its classical counterpart [54].

Currently, the fear conditioning test is considered to be one of the most interesting and informative (Fig. 2). It is based on classical conditioning with repeatedly presented, initially conditioned stimulus like a sound paired with an unconditioned aversive stimulus like a mild electric shock [49]. In repeated testing, the experimental animal normally starts to exhibit fear (freezing) only exposed to the sound [44]. This model can be used to study memory traces, or engrams that include the auditory cortex with memory traces of sounds; the hippocampus with memory traces associated with electric shock; the amigdala where the sound is paired with electric shock and the context [48, 50].

There is another variant of this test described in the literature: contextual fear conditioning [44, 45, 50]. We will discuss this variant below.

Assessment of declarative memory

Declarative memory consists of semantic memory and episodic memory. Learning and engram consolidation in the declarative memory system depend on the hippocampus and other brain structures located in the medial temporal lobes. In the studies of the hippocampus role in memory formation, spatial memory and contextual memory are assessed most frequently [45]. The finding that hippocampal principal neurons (place neurons) are activated when an experimental animal is placed in a specific environment allowed in vivo physiology to make a significant contribution to understanding the mechanisms of spatial memory formation and consolidation [45, 56].

The most widely used test for spatial memory assessment is the Morris water maze. The test was developed by R.G. Morris and has been used mostly in rats because mice are reluctant swimmers in their natural environment, which is why water tests are not suitable for them [8]. In the classic version of the test the animal is placed into an open circular pool that is filled with non-transparent water. The animal must swim to find a hidden platform that is submerged below the water surface and placed in a fixed location. The rodent gets no visible (proximal) cues to navigate to the platform when started from different, random locations around the perimeter of the tank, so must use hidden (distal) cues for spatial navigation [50].

The rodent placed in the maze for the first time will swim until it finds a hidden platform and climbs onto it. The rodent normally remembers the platform location very quickly and in the next tests spends less time for its search. In addition, once the animal realizes that the platform is an escape from the maze, it is much quicker to find the platform in different parts of the tank in subsequent test sessions. At the same time, in rodents with hippocampal lesions the place navigation is impaired: they either cannot understand the task or cannot remember the location of the platform [45].

Other tests to assess spatial deficits in the hippocampus include the radial arm maze test [57] and contextual fear conditioning [50]. A classic version of the radial arm maze consists of 8 arms radiating from a central platform. Some arms contain no food bait and refer to reference memory, the arms referring to working memory contain food bait at the beginning of the test. The correct response of the animal is an entry into a baited arm, a re-entry into a non-baited arm is an error. Then, the animal must move to a different arm to find a food bait, remembering the location of the bait each time, so that its working (short-term) and reference (long-term) memory can be assessed [51]. The main disadvantage of this test is its complexity: its protocols are quite time-consuming.

In the studies of the role of the hippocampus in memory formation, a term "cognitive map" can be encountered. It is a mental model of the environment's layout: presence and location of certain landmarks and entities, their relationship to each other within a certain time frame or event [45, 50].

In addition to examining spatial memory for the location of objects, the tests also study associative learning ability. Associative learning is an adaptive process of learning to anticipate events. One of the tools to study the mechanisms of associative learning is the contextual fear conditioning test. The variable used in the contextual and stimulated formation of the conditioned fear reflex is the freezing that follows the combination of an unconditioned stimulus (electric shock) with a conditioned stimulus. Freezing is a defensive response and is manifested by the absence of body movement (except breathing) for 0.75 s or longer [44, 47]. This test models one of the most commonly used hippocampal-related behavioral tasks that reflects learning and episodic memory formation in rodents and correlates with adult hippocampal neurogenesis rates.

Neurogenesis is the process by which new neurons are generated. There is evidence that hippocampal neurogenesis continues throughout life in many adult and even older mammals [44]. According to the modern outlook, neurons are generated in the subventricular zone of the olfactory bulb and in the dentate gyrus of the hippocampus [58, 59]. The hippocampus and hippocampal neurogenesis are essential for the formation of long-term cortical memory through consolidating

the episodic memory traces [58]. The process of the memory retrieval and expression is highly dependent on the hippocampus, but the role of the hippocampus diminishes over time, which may be related to the gradual transfer of memory traces to extrahippocampal areas, such as the neocortex. This process is supposed to be essential to free the hippocampus from outdated and unused information by storing memory traces in the cortex, thus making room for learning new things [60]. Also, hippocampal-cortical memory trace transfer allows to preserve memory traces because the constant integration of new neurons into existing neuronal networks would damage the structure of information acquired before [44]. However, the mechanisms, by which memory becomes completely dependent on the cortical structure and independent of the hippocampus, remain unknown [44, 58].

When hippocampal neurogenesis is physically or genetically suppressed, the period of hippocampus-dependent associative fear memory becomes longer [44, 58]. Inversely, adult neurogenesis enhanced by physical exercise shortens the period of the hippocampus dependent memory without loss of information. These observations paved the way for understanding of the mechanisms of the hippocampal-cortical complementary learning [44].

Thus, the study of various memory types provides the basis for the assessment of cognitive functions, neurogenesis, and learning processes.

Assessment of emotional states

Neurodegenerative diseases are often accompanied by emotional dysregulation when people exhibit inadequate emotions (for instance, in PD or AD patients) [61] or depression-like behavior (physiological ageing, etc.) Side effects of various anti-degenerative agents on the emotional state also have an impact on daily life.

To assess emotional states and depression-like behavior in mice, the forced swim test, the tail-suspension test, the sucrose preference test, and the conditioned place preference are widely used [62]. The first two tests are the most significant in preclinical studies of antidepressants [63], the third one allows to measure sensitivity to rewards.

The forced swim test (the Polsort test), was first introduced in 1977 to evaluate new antidepressants [64]. The method is based on the observation that a mouse, when forced to swim in a situation from which there is no escape, will, after an initial period of vigorous activity (swimming or climbing), eventually cease to move altogether making only those movements necessary to keep its head above water. This behavioral immobility was described as a state of despair in which the animal has learned that escape is impossible [64, 65]. Antidepressant agents have been shown to reduce the immobility time in the test. Reduction in passive behavior is interpreted as an antidepressant-like effect [66]. Another indicator of antidepressant effect is immobility latency, which is used to distinguish antidepressant from stimulant effects [67]. Administration of antidepressants prior to the test usually causes prolongation of the escape response. Different groups of antidepressants may have different effects on the behavior of rodents in the test.

The tail-suspension test induces similar behavior to the Porsolt test. The mouse hangs by its tail and its body hangs in the air [68–70]. The test is based on the assumption that the animal would try to escape the stressful situation. After some time, the animal stops struggling and becomes immobile. Longer immobility phases are the sign of depressive behavior [62]. The advantage of this test over the Porsolt test is that it eliminates the risk of water-induced hypothermia and allows the strength and energy of the animal's movement to be assessed [71].

The sensitivity to rewards can be assessed by a simple sucrose preference test in which animals have access to water with different concentrations of sucrose or without any additives, and the preference rate is then analyzed. This test is often used to assess the level of depression [62]. Reduced interest in the reward (water with sucrose) is a manifestation of depressive behavior.

The conditioned place preference test is used to assess reward behavior in rodents [72]. The test usually includes three stages. At the first stage the animal is allowed to get used to the apparatus to ascertain that there is no inherent preference for one side or the other. The amount of time required for each training session may vary depending on the stimulus (agent) being tested. The second stage is to develop a Pavlovian association between the agent and the chamber. The animal is confined in one of the chambers of the test box, each with a different pattern on the floor or the walls, and is given an addictive drug or a food bait. The third stage is to assess the reproducibility of the Pavlovian association: with repeated exposure to the chamber, the rodent prefers to spend more time on the drug-paired side of the chamber than on the food-paired side. Preference for the drug-paired side may be extinguished by repeated exposure to the chamber in the absence of reward.

Therefore, assessment of emotional states is important when working with animal models of neurodegenerative diseases, both in phenotyping animals and in the development and testing of new drugs.

Sensory and motor tests

Motor testing should be used when neurodegeneration is associated with impairments in motor activity and

walking [73, 74]. Such tests are essential in the studies [75] of PD that is characterized by significant motor impairment [76]. The group of motor tests includes the following classic tests:

1) Rotarod (rotating rod) test is used to screen new drugs for possible side effects on motor coordination or fatigue resistance in animals;
2) Motor coordination test, or the footprint test, or the catwalk test [77, 78];
3) Challenging beam test is a narrow "walking bridge" for mice to walk across to assess its sensorineural balance and coordination. The beam can vary in diameter to make the task more complicated [79].

It is also possible to use the open-field test where the number of floor line crossings is analyzed [39]. The motor coordination test and the challenging beam test are easy to perform and require no special equipment.

Sensory tests are also of interest, as age-related hearing loss occurs in one-third of adults older than 60 years and in 80 % of adults older than 85 [80]. Consequences of hearing loss may be substantial because it affects quality of life of the older people, results in functional decline, social isolation, loneliness, and the increase of depressive symptoms. Age-related hearing loss also correlates with cognitive dysfunction in the elderly, including long-term memory impairment [81]. Many studies have shown a positive correlation between hearing impairment and dementia [82], especially in AD patients.[83] Some studies have revealed that hearing impairment may be used as an early marker of cognitive decline [82]. The battery of sensory tests includes the acoustic startle test and the pre-pulse inhibition of startle, which are quite informative, but at the same time require special equipment, software, and animal training procedures.

Acoustic startle test allows to measure the mouse response to loud and sudden auditory stimuli. This test enables the assessment of a baseline startle response at various sound intensity levels as well as the reduced startle response to the repeatedly presented stimuli over time [84].

Pre-pulse inhibition of startle is an operational measure of sensorimotor gating. In this test the animal is first exposed to a low intensity stimulus, or pre-pulse (56–81 dB), followed by a subsequent stronger startle stimulus (120 dB). The pre-pulse is designed to reduce the startle response to the subsequent test stimulus; the more intense the stimulus, the greater the suppression of the startle response [85, 86].

Sensory and motor tests aid to assess the manifestations of neurodegenerative changes and to monitor either their progression or disease slowing and response to possible therapy.

Exploratory behavior tests

The open-field test and the elevated plus maze test may be useful in the studies of exploratory behavior [87, 88]. In the open-field test, only the first stage is significant in this context: when the animal is placed in the central zone of the experimental chamber, and variables such as ability to stay in the center or at the outer limits of the field, frequency of vertical activity, immobility or freezing, etc. are evaluated. If there is need to complicate the test and add other stages such as an inanimate or animate object in the center of the field, then the first stage of the test functions as a training prior the other stages of the test [86].

One of the methods to study exploratory behavior is video-recording of home cage activity during 12–24–48 hours and subsequent analysis of the images using specially developed software [78, 89, 90]. No training sessions are required in this case.

Social interaction tests

The progression of social behavioral disturbances, such as alienation or aggression, is often an important symptom of a neurodegenerative disease. Social behavioral studies assess levels of sociability, including social recognition [91], memory, and social interaction. Mice are social animals and exhibit complex social behavior in various patterns, types, and intensities of interactions [92].

The extended open-field test consists of 2 or 3 stages. The test begins with an empty box, at the second stage there is an inanimate object in the middle of the field, at the third stage — an animate object (an animal of the same or the opposite sex) [78]. The animal's interest in inanimate and animate objects indicates the level of sociability.

Currently, the three-chamber test is widely used to evaluate the sociability level or social preferences [85]. The rodent is placed in a three-chambered box with openings between the chambers. The testing includes three sessions when the behavior of the rodent is recorded: movements, freezing time, preferred chamber. During the first session, the animal is habituated to the test environment, then a previously unfamiliar and immobilized mouse is placed in one of the chambers, and finally a new social stimulus is added in the third chamber. There are various modifications of this test [78, 94].

The home cage social test is used to assess social interactions [95, 96], is inexpensive, and requires no additional equipment.

The five-trial social memory test is used to assess social recognition [97]. Over the course of multiple exposures, rodents become habituated to intruders and the inter-

action time to recognize a familiar animal decreases compared with the interaction time with a completely novel intruder. The intruders are selected from the rodents of the same age, sex, and weight, and it is mandatory that they have never encountered the test animal before. Two social stimuli are used to test the rodent: 4 short-term contacts are made with one intruder, and in the fifth trial another intruder is placed. The interaction time with the first, already familiar intruder would gradually decrease and the interaction time with the unfamiliar rodent would increase significantly.

Parental behavior and parental care are components of social behavior [98], but when working with neurodegeneration models, they are most often irrelevant and unused, so the above tests for assessing social memory and recognition are of the paramount importance.

Conclusion

The objective of this review was to summarize the knowledge of the modern tests for behavioral analysis in mice with experimentally induced neurodegeneration and to to assist in the selection of a test battery that will provide a comprehensive neurobehavioral phenotype of the animal. A careful approach to the selection of an experimental model and the necessary tests for a specific study allows the data obtained to be used both in fundamental research and in clinical practice.

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Brain–Computer Interface Using Functional Near-Infrared Spectroscopy for Post-Stroke Motor Rehabilitation: Case Series

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Abstract

Introduction. *Non-invasive brain–computer interfaces (BCIs) enable feedback motor imagery [MI] training in neurological patients to support their motor rehabilitation. Nowadays, the use of BCIs based on functional near-infrared spectroscopy (fNIRS) for motor rehabilitation is yet to be investigated.*
Objective: *To evaluate the potential fNIRS BCI use in hand MI training for comprehensive post-stroke rehabilitation.*
Materials and methods. *This pilot study included clinically stable patients with mild-to-moderate post-stroke hand paresis. In addition to the standard rehabilitation, the patients underwent 10 nine-minute MI fNIRS BCI training sessions. To evaluate the quality of fNIRS BCI control, we assessed the percentage of time during which the classifier accurately detected patient's mental state. We scored the hand function using the Action Research Arm Test (ARAT) and the Fugl-Meyer Assessment (FMA).*
Results. *The study included 5 patients at 1 day to 12 months of stroke. All the participants completed the study. All study participants achieved BCI control rates higher than random (41–68%). While three patients demonstrated the clinically significant improvements in their ARAT scores, one of them also showed an improvement in the FMA score. All the participants reported experiencing drowsiness during training.*
Conclusions. *Post-stroke patients can operate the fNIRS BCI system under investigation. We suggest adjusting the feedback system, extending the duration of training, and incorporating functional electromyostimulation to enhance training effectiveness.*

Keywords: *stroke; rehabilitation; motor imagery; brain–computer interface; near-infrared spectroscopy; neuro-bio-control*

Ethics approval. The study was conducted non-invasively in accordance with the ethics of the Declaration of Helsinki. The study protocol was approved by the Local Ethics Committee of the Research Center of Neurology (Protocol No. 5-4/22, 1 June 2022). All the participants signed informed consent.

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Conflict of interest. The authors declare no apparent or potential conflicts of interest related to the publication of this article.

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Интерфейс мозг–компьютер, основанный на спектроскопии в ближней инфракрасной области, в двигательной реабилитации после инсульта: описание серии случаев

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Аннотация

Введение. *Неинвазивные нейрокомпьютерные интерфейсы позволяют проводить тренировки представления движения с предъявлением обратной связи в двигательной реабилитации пациентов неврологического профиля. В настоящий момент практически не изучено применение интерфейса мозг–компьютер (ИМК) на основе регистрации спектроскопии в ближней инфракрасной области (БИКС) в двигательной реабилитации.*

Цель *исследования — оценить возможность применения БИКС-ИМК для проведения тренировок представления движения руки в комплексной реабилитации пациентов после инсульта.*

Материалы и методы. *В данное пилотное исследование включали клинически стабильных пациентов с постинсультным парезом руки лёгкой или средней степени выраженности. Пациенты получали 10 тренировок представления движения под контролем БИКС-ИМК, каждая длительностью по 9 мин, в дополнение к стандартной реабилитационной программе. В качестве показателя качества управления БИКС-ИМК оценивали достигнутый процент времени правильного распознавания классификатором ментального состояния пациента. Функцию руки определяли по шкалам ARAT и Фугл-Мейера.*

Результаты. *В исследование были включены и завершили его 5 пациентов с давностью инсульта от 1 дня до 12 мес. Все пациенты достигли качества управления БИКС-ИМК выше случайного (41–68%). Клинически значимое улучшение двигательной функции руки достигнуто у 3 пациентов по тесту ARAT, у одного из них — также по шкале Фугл-Мейера. В процессе тренировок все пациенты отмечали сонливость.*

Заключение. *Пациенты после инсульта способны управлять исследованной системой БИКС-ИМК. Для увеличения эффективности тренировок рекомендовано изменить сценарий предъявления обратной связи, увеличить продолжительность тренировок, включить в аппаратный комплекс функциональную электромиостимуляцию.*

Ключевые слова: *инсульт; реабилитация; представление движения; интерфейс мозг–компьютер; спектроскопия в ближней инфракрасной области; нейробиоуправление*

Этическое утверждение. Исследование выполнено неинвазивным методом в соответствии с этическими нормами Хельсинкской декларации. Протокол исследования одобрен Локальным этическим комитетом ФГБНУ «Научный центр неврологии» (заключение № 5-4/22 от 01.06.2022). Все пациенты подписали информированное согласие.

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Introduction

Brain–computer interfaces (BCIs) are systems that translate brain activity signals into commands for the output devices. Non-invasive BCIs enable feedback motor imagery (MI) training in neurological patients to support their motor rehabilitation. At present, several systematic reviews comprise the evidence base for employing BCIs, which utilize sensory and motor EEG rhythms (EEG BCIs), in post-stroke rehabilitation [1–4]. We also conducted a randomized controlled trial (RCT) to evaluate effectiveness of using EEG BCI with visual and exoskeleton-mediated kynesthetic feedback in the post-stroke population [5, 6]. A series of train-

ing sessions for MI, controlled with this technology, resulted in improved grasping and pinching. However, the EEG-BCI use is complicated due to the necessity of applying EEG gel onto the scalp, signal artifacts in patient motion and muscle contraction during training, and the low spatial resolution of signal source detection.

Functional near-infrared spectroscopy BCIs (fNIRS-BCIs) is a non-invasive BCI to conduct feedback MI training. The sources of brain activity may involve alterations in concentrations of oxy-, deoxy-, and total hemoglobin up to 4 cm below the head surface. This technology does not require the use of any electrode gel, and patient movements during training do not result in significant signal distortion. At present, fNIRS-BCI remains largely unexplored approach to post-stroke rehabilitation. The latest review [7] of biofeedback methods mentions only one fNIRS-BCI study in post-stroke rehabilitation [8]. The study protocol implied the presentation of the signal amplitude as the color and the height of the bars on the monitor rather than signal classification.

The exploratory study of this technology with assessment of the attained level of online signal detection was necessary as an initial test prior to the development of the fNIRS BCI effectiveness RCT protocol.

The study **objective** is to evaluate the potential fNIRS BCI use for customized hand MI training in comprehensive post-stroke rehabilitation.

Materials and methods

Study design

This pilot study is a case series of post-stroke rehabilitation integrating standard management with additional fNIRS BCI mental training.

The study was conducted at the Institute of Neuro-Rehabilitation and Recovery Technologies of the Research Center of Neurology from June to October 2022. The study included inpatients receiving scheduled rehabilitation. Each patient participated for a total of 12 days including five training days followed by two days off, and then five more training days. fNIRS BCI training supplemented the standard clinical rehabilitation program. Prior to the first fNIRS BCI training session and post the last one, hand motion was assessed and scored using international validated scales.

The study protocol was approved by the Local Ethics Committee of the Research Center of Neurology (Protocol No. 5-4/22, 1 June 2022). Patient participation in this study was entirely voluntary, and all participants provided informed consent. The study protocol was pre-registered in the local research project protocol database of the Institute of Neuro-Rehabilitation and Recovery Technologies (ID 210).

Inclusion criteria

Inclusion criteria: primary or recurrent stroke with a supratentorial CT or MRI-confirmed focus, mild-to-moderate clinical paresis of the distal upper limb, the stroke time of one day to 12 months, clinically stable state without any life-threatening conditions, and informed consent to participate in the study.

Exclusion criteria: severe speech, vision, and/or cognitive function impairment and/or hand tissue contractures.

Standard rehabilitation course

All patients underwent a two-week rehabilitation course that included personal instructor-led exercise therapy, neuro-muscular electric stimulation of lower limb muscles, therapeutic massage, robotic biofeedback mechanotherapy to recover hand fine movements, and stationary cycling exercises. The above activities were performed on daily basis, except for the days-off (10 sessions each).

fNIRS BCI training

MI fNIRS BCI training was performed on a daily basis except for the days-off in addition to the standard rehabilitation course. Each patient underwent 10 training sessions.

The study used a non-invasive BCI based on the recognition of a BOLD cortical signal in the hand MI expressed as changes in the relative concentrations of oxy- and deoxyhemoglobin assessed by near-infrared spectroscopy. The NIRScout system (NIRxMedicalTechnologies), with 8 detectors and 16 sources, was used for fNIRS. The study protocol, the filtration method, and the classification of brain activity signals and the software used were described previously [9]. The patient received visual feedback on the 22" computer monitor. The flow chart is presented in Fig. 1.

The investigator customized movement types for MI during BCI training and selected the most difficult (for which, accordingly, the lowest score on this test was assigned) movement based on the patient's ARAT results. Before each training session, the investigator asked the patient to repeat the target movement several times until they confirmed their readiness to perform it mentally (priming). If the movement implied manipulating an item (ARAT), the item was provided to the patient during priming.

During the procedure, a BCI cap equipped with sources and detectors was put on the patient's head. During

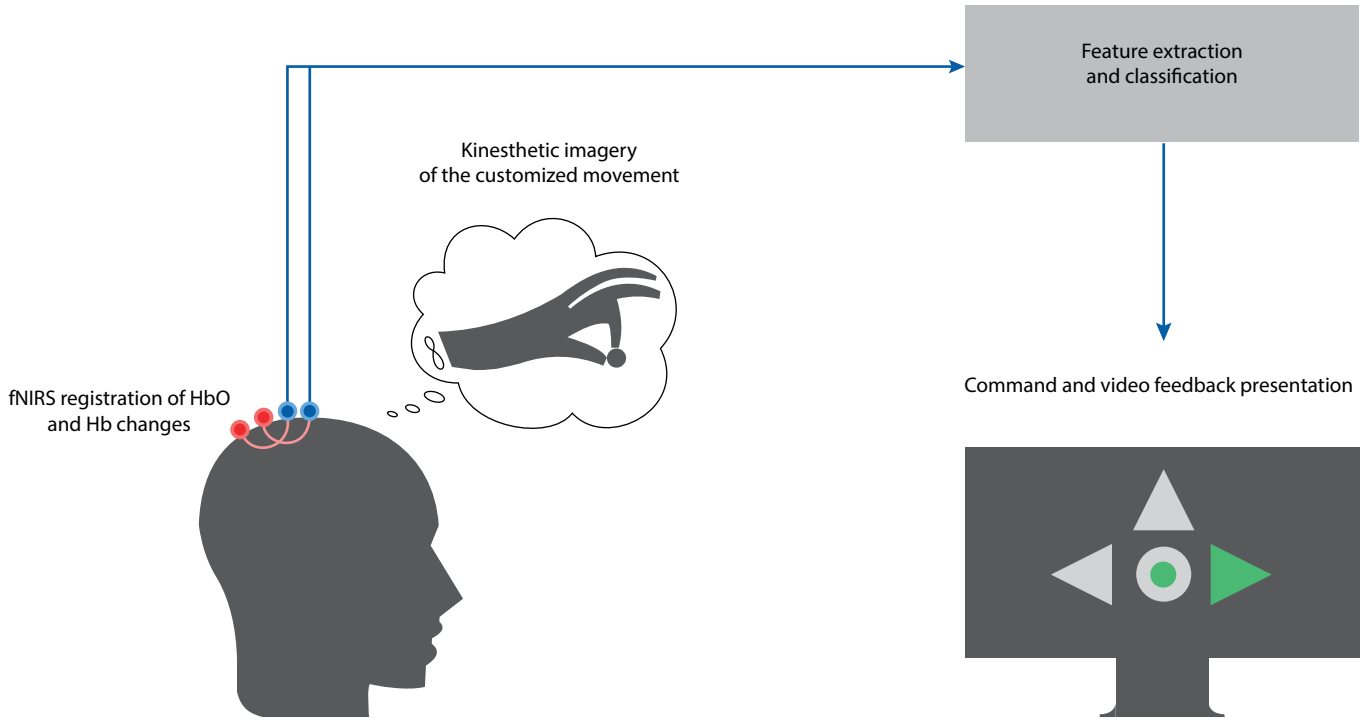

Fig. 1. The fNIRS BCI and post-priming training flow chart
Hb, deoxyhemoglobin; HbO, oxyhemoglobin.

the session, the patients sat by the computer monitor at the desk with their hands comfortably positioned on either the armrests or the desk. There was a fixation point consisting of a circular shape in the center of the dark screen to stabilize the patient's gaze. Additionally, three arrows surrounded the fixation point, which provided commands with colors that changed dynamically. Following one of three commands, the patient either kynesthetically imagined slow motion of their left or right hand (with the corresponding arrow changing color) or relaxed and directed their gaze towards the center of the screen (with the upper arrow changing color). When MI task was successfully detected by the classifier, a gaze fixation point in the center of the screen was gradiently coloring green. Following the command to relax, the point color did not change in any classifier response.

Each nine-minute training session consisted of four units, with two commands randomly given to the left and right hand for 15 seconds, interspersed with rest intervals. Two-second preparatory instruction preceded the main commands.

Signal acquisition and processing

Sources and detectors were nested on the EEG cap. Signal recording frequency was 15.625 Hz. Recorded radiation intensities were calculated as oxyhemoglobin (HbO) and deoxyhemoglobin (Hb) using the modified Beer–Lambert law. To classify active states, the signal was filtered with a first order Chebyshev type I filter with 1 dB passband ripple and 0.005 Hz cutoff frequency. To classify rest vs active state, the signal was filtered with a second order Chebyshev type I filter with 1 dB passband ripple. The cutoff frequencies were selected for zero drift at the command frequency. For classification, we used linear discriminant analysis with additional learning based on the previous units of the current session and the participant's previous test sessions. In gradual classification, we first classified resting vs. active state and left hand vs. right hand in the following classification of active states. Per-second record intervals were classified.

Endpoints

First of all, this pilot study evaluated the quality of the detection of patient's mental states during training by the classifier. This indicator is measured as the percentage of correctly detected intervals. Over 33% of the total amount of the classified intervals is considered to be higher than random because the patients performed three mental tasks ($100\% / 3 \approx 33\%$).

For the pre- and post-rehabilitation assessment of the hand function, we used the ARAT scale (with a maximum possible score of 57 points and a clinically significant increase in 6 points in the chronic stroke period and 12–17 points in the acute stroke period) [10, 11] and the Fugl-Meyer Assessment of the Upper Extre-

mity (with a maximum possible score of 126 points and a clinically significant increase in 5 points) [10, 12]. The trained physician performed the blinded movement assessment.

Statistical data processing

The resulting percentage of the detection of brain signal activity is presented as the median and the 25th and 75th quartiles. The MatLab R2019b package (MathWorks, Natick, MA, USA) was used for analysis.

Because the study was a non-comparative exploratory case series, we did not use other statistical methods.

Results

Population

While eight patients were successfully screened, five were included in the study and three were not included. Table 1 shows the profiles of the participants. All five participants completed the study with no withdrawals.

In order to train MI, we selected the following movements: 'to pinch and hold a 6-mm ball with their digit 1 and digit 4 for P1, P3, and P4; 'to pinch and hold a 6-mm ball with their digit 1 and digit 2 for P2; 'to pinch and hold a 1.5-mm ball with their digit 1 and 4 for P5.

Motor control and improvement

All patients were able to control MI fNIRS-BCI with better than random classification accuracy rate (>33%; Table 2).

Following comprehensive inpatient rehabilitation and additional MI fNIRS-BCI training, all patients showed improved motor scores (Fig. 2). While three patients demonstrated the clinically significant improvement in ARAT score, one of them also achieved significant improvement in FMA score (Fig. 2).

The MI fNIRS BCI training was not associated with any adverse events. However, all participants reported experiencing drowsiness during training, which impaired concentration and task performance by the end of the session.

Discussion

In this study, we demonstrated the potential fNIRS BCI

Table 1. Patient demographics and baseline characteristics

Patient	Sex	Age, years	Stroke type	Stroke lesion side	Stroke time, months ago	APAT score	FMA score (upper extremity)
P1	Male	71	Primary	Left	12	44	107
P2	Male	58	Primary	Left	12	39	104
P3	Male	58	Secondary	Right	8 and 2	35	114
P4	Male	49	Primary	Right	< 1	35	116
P5	Female	43	Primary	Left	1	52	122

Table 2. Resulting BCI control and motor score improvement

Patient	Classifier accuracy, %	ARAT score improvement	FMA score improvement
P1	68 [57; 73]	6*	1
P2	41 [37; 47]	4	3
P3	45 [41; 47]	6*	0
P4	45 [34; 50]	20*	9*
P5	49 [46; 59]	5	3

Note. *Clinically significant improvement as adjusted by the stroke time [10].

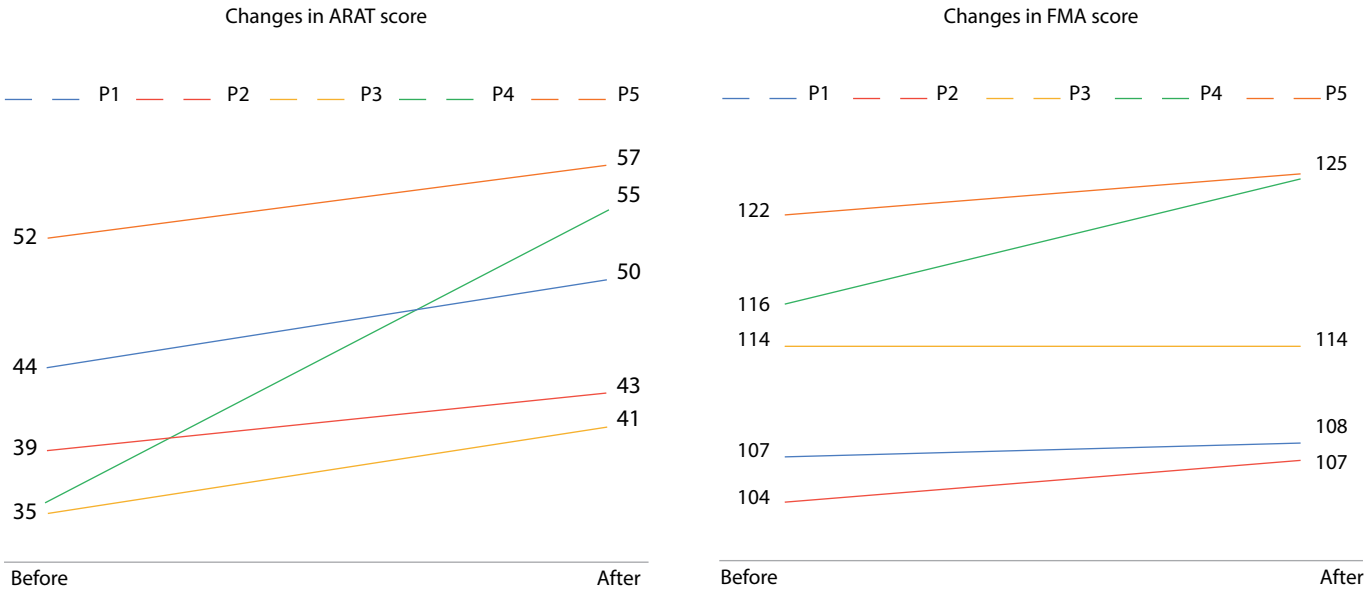

Fig. 2. Changes in motor scores during rehabilitation and additional fNIRS BCI training.

use for customized hand MI training in comprehensive post-stroke rehabilitation. All study participants achieved BCI control rates higher than random.

In the study, the total MI training exposure was 1.5 h (10 nine-minute sessions), which is significantly lower than the total training exposure of 5–27 h, with the session duration of 0.5–1.5 h, in other non-invasive BCI studies [13]. Nevertheless, patients developed drowsiness even during the shorter sessions. Thus, we find it reasonable to change the feedback scenario for play or various visualizations and to break the session units with pauses. Notably, MI drowsiness was mentioned in other studies [13].

Following the fNIRS BCI, all study participants showed improvement in motor function on at least one scale, including clinically significant improvement in three patients. However, the study design did not include a control group and the range of inclusion criteria was quite broad, which limits this exploratory study. Therefore, it is impossible to prove that the resulting improvement was caused by fNIRS BCI training. Nevertheless, the effectiveness of MI fNIRS BCI training has been demonstrated in a number of meta-analyses and systematic reviews based on multiple RCTs [1–4, 13]. Nowadays, professionals are searching for the most convenient and practical BCI systems and feedback scenarios [14].

Although the fNIRS BCI is a more convenient method than EEG BCI, it is the EEG BCI that has been evaluated in the vast majority of studies the present systematic reviews are based on. M. Mihara et al. showed fNIRS BCI effectiveness for rehabilitation of patients with subcortical stroke in the only randomized trial involving 20 patients: six 20-minute training sessions contributed to a better improvement of motor function measured as FMA score in the active group than in the sham fNIRS BCI group [8]. Interestingly, unlike the system we used in our study, the fNIRS BCI technology used by M. Mihara et al. did not involve any online signal classification. Therefore, additional, unobvious actions are needed to translate the recorded signals into commands to start exoskeleton operation or electrostimulation, if these methods are used for additional sensorimotor feedback.

The hallmark of our study is customisation of the movement type for further in-training MI based on the ARAT. This approach corresponds to the modern concept of customized rehabilitation and allows the use of goal attainment scaling [10].

A recent meta-analysis showed a better effect of BCI systems with functional electrostimulation in rehabilitation setting, as compared to those with the exoskeleton for kinesthetic feedback or with visual feedback only [13]. Therefore, we recommend to supplement the fNIRS BCI hardware with functional electromyostimulation controlled by brain activity signals in the MI during further rework.

Conclusion

In this pilot study, we demonstrated the potential fNIRS BCI use for customized hand MI training in comprehensive post-stroke rehabilitation and identified the ways to improve this technology and the training protocol.

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Relapsing Autoimmune GFAP Astrocytopathy: Case Report

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Abstract

Introduction. *Glial fibrillary acidic protein (GFAP) is the main component of intermediate astrocyte filaments. In 2016, anti-GFAP antibodies (Ab) were identified as the specific biomarker for the first established CNS inflammatory disorder subsequently called autoimmune GFAP-astrocytopathy (A-GFAP-A). Since GFAP is localized intracellularly, GFAP Ab do not appear to be directly pathogenic though serve as a biomarker of immune inflammation. Although presence of GFAP-Ab in the serum (but not in the CSF) could be observed in various CNS immune-mediated diseases, detection of GFAP-Ab in CSF is only characteristic for A-GFAP-A. A-GFAP-A usually develops after the age of 40 and mostly manifests acutely or subacutely with symptoms of meningoencephalomyelitis or its focal forms. Linear perivascular radial cerebral white matter enhancement is a specific MRI finding of A-GFAP-A. Concomitant neoplasms or autoimmune disorders, as well as co-expression of other antineuronal antibodies are not uncommon in A-GFAP-A. Usually, disease responds well to immunotherapy, and prolonged remission could be achieved, however recurrent disease course and fulminant cases are also described in the literature. In these cases, long-term immunosuppression is required. Data on epidemiology, etiological factors, and precise pathogenesis of A-GFAP-A are still limited. Due to the lack of long-term follow-up data, diagnostic criteria, generally accepted treatment strategies or prognostic risk factors for relapse and outcome of the disease have not yet been established and precised. We present the first description of a case of relapsing A-GFAP-A in Russia and an analysis of the current data on the pathogenesis, clinical features, as well as the diagnostic challenges and treatment approaches for A-GFAP-A.*

Keywords: *GFAP; glial fibrillary acidic protein; autoimmune GFAP astrocytopathy; autoimmune encephalitis; meningoencephalitis; meningoencephalomyelitis*

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Клинический случай рецидивирующей аутоиммунной GFAP-астроцитопатии

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Аннотация

Введение. *Глиальный фибриллярный кислый белок (GFAP) является ключевым компонентом промежуточных филаментов астроцитов. В 2016 г. антитела к GFAP (GFAP-АТ) были идентифицированы в качестве специфичного биомаркера впервые установленного воспалительного заболевания ЦНС, которое назвали аутоиммунной астроцитопатией, ассоциированной с GFAP-АТ (A-GFAP-A). Поскольку GFAP локализован внутриклеточно, непосредственно GFAP-АТ, по-видимому, не патогенны, но служат биомаркером иммунного воспаления. Диагностическая ценность обнаружения GFAP-АТ в цереброспинальной жидкости выше, чем в сыворотке крови, поскольку изолированное выявление GFAP-АТ в крови (но не в цереброспинальной жидкости) может наблюдаться и при других иммуноопосредованных заболеваниях с поражением центральной нервной системы. A-GFAP-A обычно поражает лиц старше 40 лет и в большинстве случаев проявляется острым или подострым развитием симптомов менингоэнцефаломиелита или его ограниченных форм. Характерным для A-GFAP-A МРТ-признаком является линейное периваскулярное радиальное контрастное усиление в белом веществе полушарий головного мозга, локализующееся перпендикулярно по отношению к желудочкам. Сопутствующие новообразования или аутоиммунные расстройства, а также ко-экспрессия с антинейрональными антителами — не редкость при A-GFAP-A. Заболевание, как правило, хорошо поддается иммунной терапии, хотя рецидивирующее течение, требующее длительной иммуносупрессии, и единичные случаи летального исхода также имеют место.*

Сведения об эпидемиологии, этиологии и патогенезе A-GFAP-A ещё достаточно ограничены. В связи с отсутствием данных долгосрочного наблюдения диагностические критерии, общепринятые схемы лечения, прогностические факторы для оценки риска рецидива и исхода заболевания не установлены. В статье представлено первое в России описание клинического случая рецидивирующей A-GFAP-A, а также приведён анализ литературы с освещением накопленных к настоящему времени знаний о патогенезе, клинической картине, а также трудностях диагностики и лечения A-GFAP-A.

Ключевые слова: *GFAP; глиальный фибриллярный кислый белок; аутоиммунная GFAP-астроцитопатия; аутоиммунный энцефалит; менингоэнцефалит; менингоэнцефаломиелит*

Этическое утверждение. Исследование проводилось при добровольном информированном согласии пациента, в том числе на публикацию клинического случая.

Источник финансирования. Авторы заявляют об отсутствии внешних источников финансирования при проведении исследования.

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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Introduction

In 2016, the researchers led by V. Lennon (Mayo Clinic, Rochester, MN, USA) presented a novel autoantibody (Ab) to the cytosolic protein of intermediate astrocyte filaments. As its antigen he identified glial fibrillary acidic protein (GFAP), the main protein of intermediate astrocyte filaments, that is crucial for keeping them morphologically stable, forming the blood–brain barrier, and regulating the synapse function [1, 2]. Testing with tissue-based (TBA) and cell-based (CBA) assay of over 100,000 serum and/or cerebrospinal fluid (CSF) samples found anti-GFAP Ab in 103 patients of those with suspected autoimmune neurological disorder [1]. Retrospective analysis of the relevant medical records showed that most of the seropositive patients clinically had glucocorticosteroid-sensitive menyngoencephalitis with or without concomitant myelitis, which resembles nonvasculitic autoimmune inflammatory meningoencephalitis as described earlier [3, 4]. Thus, they introduced a new clinical entity called autoimmune GFAP-meningoencephalitis (or A-GFAP-A) that is distinct from other conditions typically considered in the differential diagnosis including infections, granulomatoses, canrcinomatosis, demyelinating diseases, and CNS vasculitis and lymphoma. Further studies proved specific CSF GFAP seropositivity to establish A-GFAP-A [5–7].

In this paper, we present first known A-GFAP-A clinical case in Russia.

Clinical case

Patient M., male, 66 y.o., who had mild COVID-19 in January 2021, was immunized with bicomponent Sputnik-V in April–May 2021. In mid-May, the patient traveled to Thailand where he experienced significant general fatigue, daytime drowsiness, and decreased appetite. Upon his return to Moscow on 5 July 2021, his wife found him spatially and temporally disoriented, hallucinating, and feverish with a body temperature elevated up to 38ºC (100.4ºF). Symptoms that developed over the next two weeks included ascending numbness of lower limbs (up to the costal arch), impaired coordination, and frequent urination, later acute urine retention. The patient was admitted to the hospital and underwent trocar cystostomy. His condition gradually deteriorated, with short-term memory loss, loss of independent mobility, and episodes of emotional agitation.

The MRI of the brain and the cervical spinal cord (July 2021) showed multiple T2-hyperintense lesions in the periventricular white matter of the hemispheres, the brainstem, and the cervical spinal cord with signs of activity: contrast enhancement of the periventricular lesions and the lesion at the level of C2 vertebra on the post-contrast T1-weighted images (T1+C). The MRI image was interpreted as a demyelinating process. Blood and CSF tests excluded neurosarcoidosis, neuromyelitis optica spectrum disorder (NMOSD) with anti-aquaporin-4 (AQP-4) Ab, herpes simplex encephalitis, and tuberculous encephalopathy. The diagnosis of multiple sclerosis relapse was established. The patient received pulse regimen of IV dexamethasone (total dose 144 mg) and symptomatic alimemazine and hydroxyzine with suboptimal improvements (relief of positive psychotic symptoms, improved alertness, standing with assistance).

One month later, the patient was readmitted to the hospital due to residual psychoneurological deficits. *Brain*

and spinal cord MRI (August 2021) found multifocal T2-hyperintense lesions in the brain (unchanged as compared to the previous imaging) and along the entire length of the spinal cord. T1+C images showed diffuse contrast enhancement (dirty-appearing white matter) along the centrum semiovale perivascular spaces, focal enhanced lesion in the brainstem, and multiple enhanced lesions along the entire length of the spinal cord. MRI abnormalities were interpreted as inflammatory process (suspected vasculitis). Instrumental and laboratory tests excluded CNS damage due to systemic vasculitis, primary CNS angiitis, antineuronal Ab-associated encephalitides, myelin oligodendrocyte glycoprotein (MOG) associated disease, and West Nile, dengue, chikungunya, and Japanese viral encephalitides. The patient's diagnosis was reviewed as vasculitis with affected cerebral and spinal small veins and secondary inflammatory changes in the white matter of the hemispheres, the brainstem, and the spinal cord. The patient received pulse regimen of IV methylprednisolone (total dose 5,000 mg) with pronounced improvements (steadier gait, independent mobility, totally regressed cognitive impairment, better urinary control, that allowed to remove cystostomy catheter). PO prednisolone (80 mg QD) with tapering was indicated.

Two months after the discharge (October 2021), the patient, who then received tapered prednisolone (20 mg QD), had a relapse manifested as increasing general fatigue, daytime drowsiness, cognitive impairment, and unsteady gait. *Brain and spinal cord MRI (October 2021)* showed negative changes including the increased number of T2-hyperintense lesions in the deep and periventricular white matter and more intense contrast enhancement in the hemispheric white matter, in the brainstem (primarily the pons and the middle cerebellar peduncles), and the intramedullary lesions (Fig. 1). The patient received pulse regimen of IV methylprednisolone (total dose 5,000 mg) with significant symptom regression. PO prednisolone (80 mg QD) with further tapering was indicated.

The patient's condition was stable during the next year. Control *brain and spinal cord MRI (December 2021)* demonstrated positive changes (Fig. 2). *Brain MRI (April 2021)* demonstrated no changes.

In October 2022, the patient who then received tapered methylprednisolone (8 mg QD) again had subfebrile fever, increasing daytime drowsiness, and unsteady gait. The patient was therefore admitted to the Research Center of Neurology.

Comorbidities: type 2 diabetes, stage 2 hypertension, cardiovascular risk 4, and prostatic hypertrophy.

Neurological status: The patient is fully oriented. Mild cognitive impairment (MoCa 24/30 points; with short-term memory mostly impaired). No meningeal signs. No cranial nerve abnormalities. No obvious pareses. Normal limb muscle tone. Brisk tendon reflexes (L>R). Bilateral positive Babinski sign, Rossolimo's hand sign, and palmomental reflex. Coordination tests with intention tremor or dysmetria (R=L). The patient is unstable in the Romberg test. Painless voluntary urination. Predisposition to constipation. Decreased vibration sensitivity on the knee joints and no vibration sensitivity on the ankle joints and the shinbones (L=R). Ataxic gait on wide base.

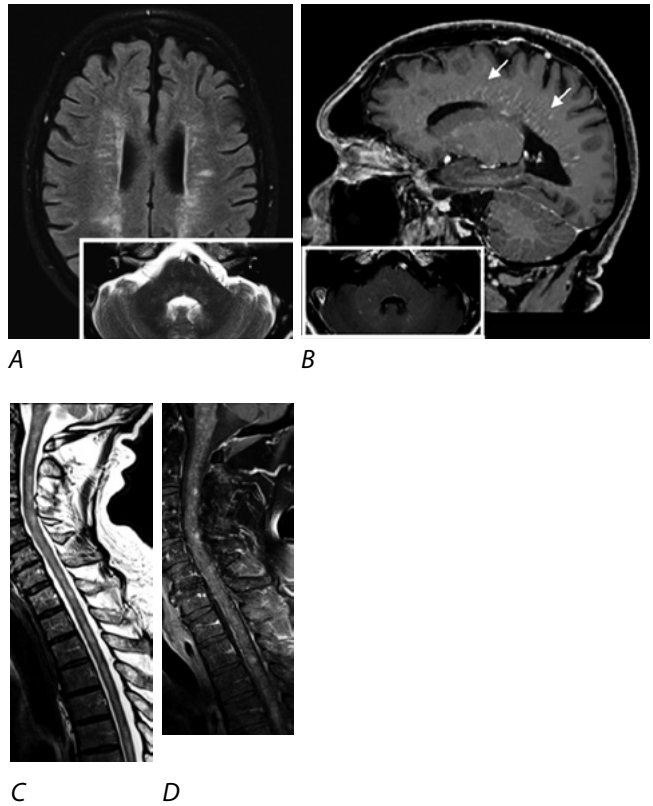

Fig. 1. Patient M.'s brain and spinal MRI (October 2021).
A — T2/T2-FLAIR: multiple hyperintense white matter lesions in the cerebral hemispheres (elongated and perivascular), the pons, the medulla oblongata, and the middle cerebellar peduncles; *B* — T1+C: linear radial perivascular contrast enhancement in the white matter of the cerebral hemispheres, the brainstem, the cerebral peduncles, and the cerebellum; *C* — T2/T2-STIR: multiple ill-defined hyperintense lesions along the entire length of the spinal cord; *D* — T1+C: focal heterogeneous contrast enhancement in the spinal cord.

Test results:
- CSF analysis (July 2021): cytosis 80/3; lymphocytes (reference: 0–10), protein 0.84 g/L (reference: 0.15–0.45), glucose 1.7 mmol/L (reference: 2.2–3.3);
- CSF analysis (August 2021): cytosis 155/3; lymphocytes, protein 0.599 g/L, normal glucose level;
- oligoclonal IgG bands in serum and CSF (July 2021): type 3;
- oligoclonal IgG bands in serum and CSF (August 2021): type 2;

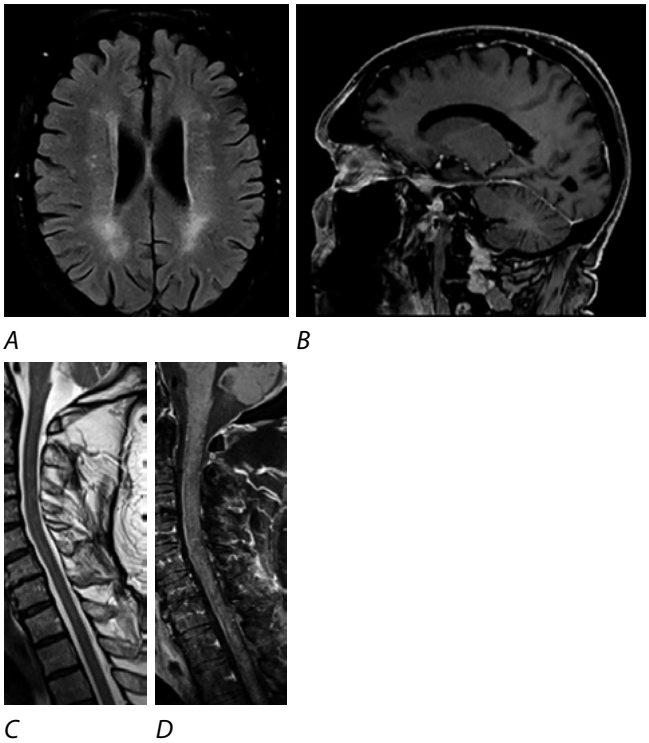

Fig. 2. Patient M.'s brain and spinal cord MRI (December 2021).
A, C — T2-FLAIR/T2-STIR: partially resolved focal changes in the brain and spinal cord. *B, D* — T1+C: no abnormal enhancement in the brain matter; decreased volume and intensity of abnormal enhancement in the spinal cord.

- no PCR DNA of the Mycobacterium tuberculosis, type 1 and/or 2 herpes simplex virus, the cytomegalovirus, and/or the Epstein—Barr virus in CSF (July 2021);
- no antibodies to West Nile, dengue, chikungunya, and/or Japanese encephalitis viruses (IgM) in serum and CSF (October 2021);
- no antibodies to neuronal antigen (IgG) inserum and CSF;
- no anti-neutrophil cytoplasmic antibodies (IgM) and/or antibodies to extractable nuclear antigens (IgG);
- no AQP-4 Ab;
- no MOG Ab;
- normal ACE activity;
- blood electrolytes (July 2021): sodium 121 mmol/L (reference 130–157), normal potassium level;
- blood electrolytes (August 2021, October 2021): normal sodium and potassium levels;
- chest CT: post-inflammatory changes in the left upper lobe and the right middle lobe. No other abnormalities.
- brain MRI (November 2022) compared to April 2021 MRI showed negative changes including enlargement of diffuse T2 hyperintense lesions in the deep white matter of both cerebral hemispheres, the brainstem, and both cerebellar hemispheres, which are elongated and perivascular with intense contrast enhancement (Fig. 3);

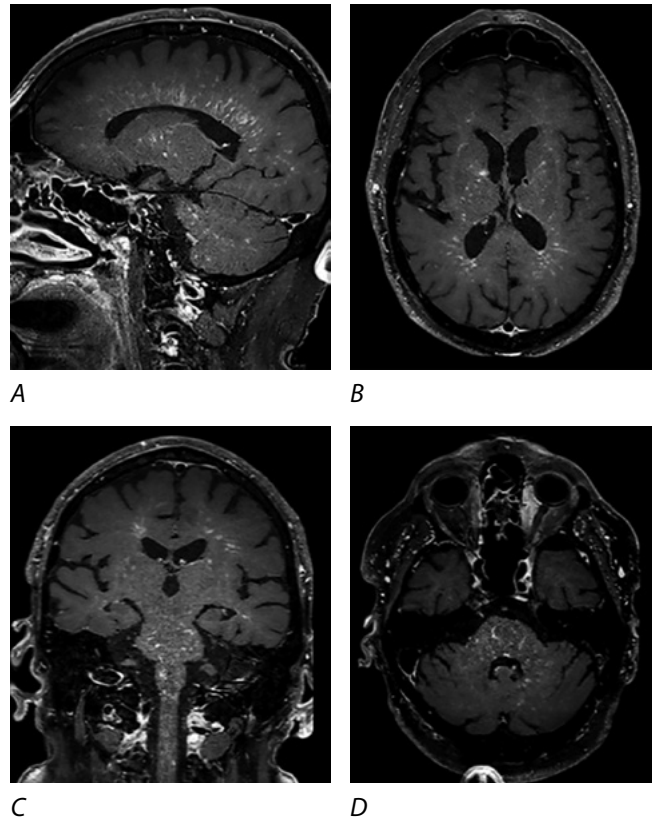

Fig. 3. Patient M.'s brain MRI (November 2022).
T1+C: contrast enhancement in the periventricular and deep white matter of the cerebral hemispheres (*A–C*), the brainstem (*C, D*), the cerebral peduncles (*D*), and both cerebellar hemispheres (*A, D*).

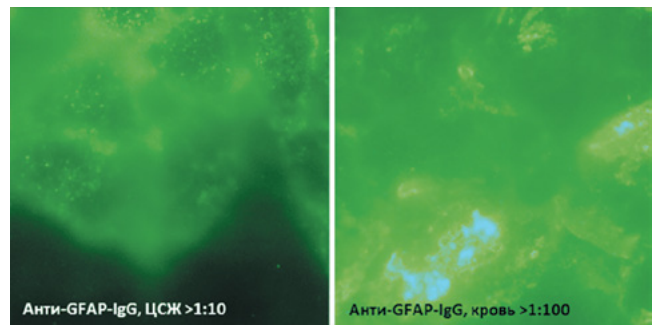

Fig. 4. GFAP Ab (IgG; indirect immunofluorescence) both in patient M.'s CSF (*A*) and serum (*B*) (November 2022).

- CSF analysis: cytosis 129/3; protein 0.75 g/L, normal glucose level;
- GFAP Ab (IgG; indirect immunofluorescence) both in CSF (Fig. 4, A) and serum (Fig. 4, B).

Based on the clinical presentation of relapsing encephalomyelitis, neuroimaging findings, and GFAP Ab in the patient's CSF and serum, we established A-GFAP-A diagnosis.

The patient received pulse regimen of IV methylprednisolone (total dose 5,000 mg). Considering the relapsing disease, we initiated anti-B-cell therapy (rituximab 1,000 mg ×2 with a 2-wk interval) with positive changes including relieved drowsiness and reversed vestibulo-atactic syndrome. We prescribed PO methylprednisolone (16 mg QD) for 1 month with tapering and eventual discontinuation. We recommended neurologic and endocrinologic follow-up, continued rituximab 1,000 mg twice a year, and PET/CT with FDG tracer to rule out paraneoplastic disease.

Discussion

GFAP is the fourth glial autoantigen with proven clinical significance after AQP-4, MOG, and SOX-1 [8]. Since GFAP is an intracellular (cytoplasmic) antigen, GFAP-specific cytotoxic T-cells must be crucial for the immune response while GFAP Ab must be a GFAP-autoimmunity diagnostic biomarker rather than a pathogen [6, 9].

A-GFAP-A may be triggered by neoplasms, conditions associated with T-cell function dysregulation (including HIV infection and use of immune checkpoint inhibitors), and history of infections [5–7, 9, 10]. Association between infections and A-GFAP-A is not fully understood. However, many patients (30–40%) have symptoms of systemic inflammation (subfebrile fever, rhinorrhea, sore throat, cough) within 1 month before the onset of CNS signs and symptoms [5–7, 11, 12]. Additionally, we aware of cases of A-GFAP-A deve loped post herpetic infections (Herpes simplex virus, Varicella zoster virus) [5, 11, 13]. Patient M. also had influenza-like prodromal syndrome. Besides, epidemiology data on symptom onset during Thailand travel and the recent history of COVID-19 do not allow us to exclude an infection as a possible trigger of disease development despite the negative results of CSF test for a wide range of infections.

Over a quarter of patients with A-GFAP-A have a past or present history of neoplasms, with ovarian teratoma accounting for almost half of these neoplasms. In addition, adenocarcinomas and carcinomas of almost all organs have been reported [5–7, 11, 14, 15]. In the case presented, routine cancer screening revealed no malignancy. Relapsing disease and the fact that over half of the neoplasms in patients with A-GFAP-A can be prospectively predicted two years after the onset of neurological symptoms [5, 6] emphasize the need for cancer suspicion in our patient.

Immune-mediated A-GFAP-A origin may be also proved by high prevalence of concomitant autoimmune conditions including type 2 diabetes (as in patient M.), psoriasis, thyroiditis, rheumatoid arthritis, myasthenia gravis, ulcerative colitis, and focal alopecia as well as reports on co-expression of antineural/glial Ab (such as NMDAR, $GABA_AR$, and AQP-4 Ab) in patients with A-GFAP-A [6, 7, 16, 17].

Although A-GFAP-A has been reported in patients ranging in age from 2 to 103 years, it is typically found in middle-aged patients (44–50 years). A-GFAP-A is equally prevalent in males and females, although paraneoplastic teratoma cases predominate in females [5–7, 15, 18]. The disease usually has an acute or subacute onset (<2 months). The most common A-GFAP-A clinical phenotypes include meningoencephalitis and encephalitis (44–61%) followed by (meningo)encephalomyelitis (11–32%), with much rarer isolated myelitis (2–11%) and meningitis (1–9%) [5–7]. The most common manifestations include altered and impaired consciousness, cognitive impairment (primarily worsened executive functions and short-term memory), mental, meningeal, vestibulo-atactic, myelopathic, and brainstem symptoms, autonomic dysfunction, and heterogeneous visual disturbances [5–7, 11, 14, 19]. Slightly less common manifestations include epilepsy, urinary dysfunction, parkinsonism, movement disorders (such as tremor, myoclonus, dystonia, chorea, and hyperekplexia), *area postrema* syndrome, and peripheral nervous system involvement [12, 20–24]. This case combined typical A-GFAP-A manifestations and gross urinary dysfunction probably caused by extensive spinal (including caudal) involvement and excessive daytime drowsiness probably caused by diencephalic involvement.

Blood tests showed hyponatremia at onset (and further normal sodium levels). This is consistent with literature data on the prevalence of hyponatremia in more than half of patients with A-GFAP-A [17]. Hyponatremia origin remains unclear. Almost all patients with A-GFAP-A demonstrate inflammatory CSF analysis pattern. The analysis typically indicates lymphocytic pleocytosis (average: 60–225/μL), with an increase in lymphocytes (mostly), monocytes, and neutrophils, and an elevated protein level (average: 0.75–2.00 g/L) [5–7, 14]. Intrathecally synthesized oligoclonal IgG is found in 42–77% of patients [6, 7, 14]. Interestingly, ca. 15% of the A-GFAP-A population demonstrate decreased CSF glucose levels at normal serum glucose levels. Underlying mechanism and clinical significance of the phenomenon are unclear. Hypoglycorrhachia combined with meningeal symptoms and MRI findings of meningeal contrast enhancement in patients with A-GFAP-A may lead to an erroneous diagnosis of infectious (primarily tuberculosis) meningitis [25, 26]. Lymphocytic pleocytosis, hyperproteinarchy, and oligoclonal IgG intrathecal synthesis were documented in all CSF analyses of patient M. during 1.5 years of follow-up. The CSF glucose level was decreased in the initial clinical episode (CSF PCR did not find Mycobacterium tuberculosis DNA), while repeat testing in one month showed a normal glucose level.

Both CSF Ab and serum GFAP Ab are important for A-GFAP-A diagnosis. However, GFAP-seropositive CSF, as more sensitive and specific, is crucial for A-GFAP-A diagnosis [1, 5–7]. Serum GFAP Ab (without CSF GFAP Ab) may be co-expressed in other immune-mediated CNS diseases (autoimmune encephalitides, NMOSD, multiple sclerosis, acute disseminated encephalomyelitis), although significance of this phenomenon is to be determined [15, 17, 27]. Because GFAP is the cytosolic protein of intermediate astrocyte filaments, its Ab detection methods are limited. The method of cellular antigen presentation followed by immunofluorescence visualization of GFAP Ab (indirect immunofluorescence) is the main method used to detect GFAP Ab [5–7]. In this clinical case, GFAP Ab were positive in both serum and CSF, which, combined with clinical and neuroimaging findings, allowed us to diagnose A-GFAP-A.

MRI shows changes in the brain and the spinal cord in most patients with A-GFAP-A. Multiple lesions are most commonly localized in the periventricular white matter, slightly less commonly in the brainstem (including the *area postrema*), in the basal ganglia, in the deep and subcortical white matter, and the spinal cord. Additionally, the cerebellum and the meninges may be involved [6, 7, 16]. T2/T2-FLAIR-hyperintense changes of the brain matter may be multifocal and confluent and mimic the MRI pattern of leukodystrophy or demyelination (especially in the presence of intramedullary and contrast-enhanced lesions) [6, 28, 29]. Thus, at the disease onset, the MRI findings of patient M. were interpreted as active demyelination, which, together with type 3 oligoclonal IgG synthesis and exclusion of neurosarcoidosis and viral encephalitis, led to the erroneous diagnosis of multiple sclerosis.

A-GFAP-A intramedullary lesions may be localized in any part of the spinal cord including the conus medullaris. In over 80% of cases, it is represented by longitudinally extensive transverse myelitis (≥ 3 adjacent spinal segments) involving gray matter and mostly centrally localized [6, 7, 30]. The presented clinical case was characterized by similar involvement of the matter along the spinal cord.

Approximately two thirds of A-GFAP-A cases demonstrate abnormal contrast enhancement in T1+C images, even sometimes with normal T2/T2-FLAIR. There may be focal, heterogeneous, leptomeningeal, and ependymal abnormal contrast enhancement and enhancement of cranial nerves and GFAP-enriched areas that are adjacent to the central canal of the spinal cord in patients with A-GFAP-A [6, 7, 30, 31]. However, linear perivascular radial contrast enhancement in the white matter of the cerebral hemispheres is the most typical and the most common (30–55%) pattern in A-GFAP-A patients [6, 7, 14, 16]. This pattern is not pathognomonic for A-GFAP-A and may be found in patients with lymphomatoid granulomatosis, intravascular lymphoma, neurosarcoidosis, and CNS vasculitis including patients without cerebral infarctions and with established angiographically negative primary vasculitis of CNS small vessels [32–35]. Some of the described cases of 'small-vessel vasculitis' are supposedly A-GFAP-A cases [6].

Linear perivascular radial contrast enhancement in T1+C images, normal 3D-TOF MR angiography findings, the inflammatory CSF analysis profile, and good response to pulse regimen of IV methylprednisolone led to the diagnosis of vasculitis involving small vessels of the brain and the spinal cord in patient M. Noteworthily, in A-GFAP-A patients with initially normal MRI findings, characteristic abnormalities may be found on subsequent scans, sometimes even after immune therapy [25]. However, in most cases, abnormal contrast enhancement in T1+C images and, less commonly, hyperintense changes in T2/T2-FLAIR partially or fully resolve following immune therapy as in patient M.

Available data on A-GFAP-A management and outcomes are based on observational and retrospective studies [5–7, 11, 14]. No prospective controlled studies were conducted, and thus no routine therapy protocols have been developed. Acute A-GFAP-A management includes standard options for immune-mediated neurological conditions such as IV high-dose methylprednisolone (IVHDMP), IV human immunoglobulin (IVIG), and high-volume plasma exchange (HVPE).

In most cases immune therapy used to manage acute disease results in evident clinical benefits. J. Xiao et al. meta-analysis that included 324 A-GFAP-A, patients showed that the patients who received only IVHDMP, IVHDMP+IVIG, and only HVPE demonstrated approximately similar response to treatment ($p = 0.769$) [14], which allows clinicians to choose any therapeutic regimen based on clinical severity, comorbidities, and financial issues of the patient.

Immune therapy may be sufficient as acute management of monophasic A-GFAP-A course. However, relapsing disease requires long-term immunosuppression in 20–50% patients [5–7, 11, 36]. Relapses often develop in decreasing doses of PO steroids usually prescribed for short terms following acute immune therapy. Two relapses in patient M. can be also explained by too early decrease of prednisolone dose. Mycophenolate mofetil, azathioprine, rituximab, or cyclophosphamide are recommended in relapsing or refractory A-GFAP-A although meta-analysis showed that azathioprine is less effective for relapse prevention than other options [14].

In this clinical case we selected rituximab due to rapid onset of its action as long-term immune therapy, which allowed us to use rituximab for management of acute A-GFAP-A when the patient was admitted to our Center with his third symptomatic relapse and to refuse from the long-term PO prednisolone use that would have been necessary following cyclophosphamide, mycophenolate mofetil, or azathioprine therapies, which is especially important in patients with concomitant diabetes. Another cause of relapsing disease may be paraneoplastic disease origin, and we recommended to patient M. PET/CT with FDG tracer to rule it out.

If timely and adequately managed, the prognosis for most patients with A-GFAP-A is good. As documented after the long-term follow-up (median 20 months) of 38 patients, the mean score on the modified Rankin scale was 1 [6] although poor response to immune therapy and significant remnant neurological deficit are also possible [5, 36]. A series of 22 clinical cases included 2 fatal outcomes in the patients who refused immunotherapy [7].

Conclusion

The presented A-GFAP-A clinical case and diagnostic search clearly illustrate the potential challenges physicians may face when examining such patients. A-GFAP-A is difficult to differentiate from other immune-mediated disorders and infectious diseases. Clinical findings and results of laboratory tests that mimic those found in patients with infectious (primarily tuberculosis) meningitis or CNS demyelinating diseases (NMOSD, multiple sclerosis, acute disseminated encephalomyelitis) often delay correct diagnosis and initiation of specific therapy, thus contributing to a worse prognosis. Further studies are needed to better understand A-GFAP-A, to develop its diagnostic criteria and therapeutic algorithms, as well as to increase awareness of medical professionals and GFAP Ab testing capacities in Russia.

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A Clinical Case of Corticospinal Tract Reorganization of Supplementary Motor Area in a Child After Acute Hypoxic Brain Injury

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Abstract

We present clinical observation of a 3-year-old child during recovery after acute hypoxic brain injury (freshwater drowning). Using diagnostic transcranial magnetic stimulation and magnetic resonance tractography with reconstruction of the corticospinal tract (CST) originated from the primary motor cortex and supplementary motor area (SMA), we determined that hypoxic brain injury induced activation of CST from the SMA. The period of reorganization was associated with the development of epileptiform patterns, that confirms the transient hyperexcitability of cortical neurons. Our findings indicate no recovery of motor function after acute hypoxic brain injury when CST originated only from SMA.

Keywords: *corticospinal tract; supplementary motor area; hypoxic encephalopathy; transcranial magnetic stimulation; motor evoked potential*

Ethics approval. The study was conducted with the informed consent of the legal representatives of the patient.

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Клинический случай реорганизации кортикоспинального тракта дополнительной моторной зоны при постгипоксическом поражении центральной нервной системы у ребёнка

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Аннотация

Представлено клиническое наблюдение ребёнка 3 лет в восстановительном периоде после перенесённого острого гипоксического состояния (утопление в пресной воде). Проведены диагностическая транскраниальная магнитная стимуляция и магнитно-резонансная трактография с реконструкцией кортикоспинального тракта (КСТ) от первичной моторной коры и дополнительной моторной зоны (ДМЗ). Установлено, что постгипоксическое поражение мозга привело к активации КСТ от ДМЗ, а период реорганизации сопровождался возникновением эпилептиформных паттернов, подтверждающих временную гипервозбудимость кортикальных нейронов. Полученные данные свидетельствуют об отсутствии у ребёнка восстановления моторной функции в восстановительном периоде острого постгипоксического состояния при наличии КСТ только от ДМЗ.

Ключевые слова: *кортикоспинальный тракт; дополнительная моторная зона; постгипоксическое состояние; транскраниальная магнитная стимуляция; моторный вызванный потенциал*

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Introduction

Transcranial magnetic stimulation (TMS) can be used for non-invasive and painless assessment of the corticospinal tract (CST) development in children in health and disease. The diagnostic potential of the TMS in the investigation of the perinatal CST damage following stroke and cerebral palsy in children has been described [1]. The role of supplementary motor area (SMA) as a reserve motor control zone becomes more important in case of disrupted cortical motor regulation [2, 3]. However, it is extremely difficult to obtain evidence for this in the clinical setting.

We present a clinical case of CST development from the SMA during recovery after acute hypoxic brain injury in a 3-year-old child.

Clinical Case

Female patient S., 3 y. 2 mos.o., was admitted to the Rehabilitation Department, the Research Institute of Children's Emergent Surgery and Traumatology, on Day 50 after acute hypoxic brain injury (freshwater drowning).

According to her history, the child fell into the swimming pool and remained face down in the water for 10 minutes until her mother noticed the girl. The mother unsuccessfully tried to resuscitate her child and delivered her to the hospital where the girl was admitted to the ICU. The patient was weaned from the ventilator and breathed spontaneously via tracheostomy. After the patient's condition stabilized, her transfer and readmission to the Research Institute of Children's Emergent Surgery and Traumatology was approved.

Neurological examination:
- vegetative state;
- tetraparesis;
- bulbar palsy;
- Disability Rating Scale (DRS) score, 24 points;
- Bykova–Lukyanov Scale of Communication Activity score, 21 points (poor rehabilitation prognosis) [4, 5].

On Day 56 from injury, we performed standard diagnostic TMS using the Neuro-MS Monophasic magne tic stimulator and the Neuro-MEP-Micro 2-channel myograph (Neurosoft, Ivanovo, Ivanovo Region, Russian Federation), with a 9-cm circular coil. Stimulation area was localized with single stimuli in F3 projection for the left hemisphere and in F4 projection for the right hemisphere on 10–20 international scheme (intensity ≥ 50%) contralaterally to the side of motor evoked potential (MEP) registration.

The electric current in the circular coil was directed clockwise for the left hemisphere and counterclockwise for the right hemisphere. Disposable surface electrodes were placed on *m. Abductor pollicis brevis* projection bilaterally in accordance with the contralateral recording scheme [6]. The coil was moved by 1 cm to determine stimulation area if MEP was evoked, and motor threshold was assessed using 10–20% power of stimulus increment according to the to the Rossini-Rothwell algorithm [7]. We assessed MEP parameters including motor threshold, latency, amplitude, and shape.

No reliable MEP was recorded with stimulation in the 50–85% range of stimulus intensity, and only a stimulation artifact was detected.

On the same day, we performed neuroimaging, i.e. magnetic resonance (MR) tractography with CST reconstruction arising from primary motor cortex (M1) and SMA using the Philips Achieva dStream 3.0T scanner and MR Fiber Trak software on the IntelliSpace Portal.

Only single projections originated from SMA were seen in the left hemisphere (Fig. 1).

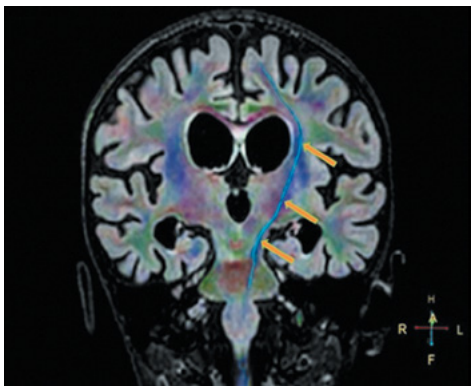


Fig. 1. MR tractogram with CST reconstruction of patient S. on day 56 from injury.
Single CST projections from SMA (blue) are seen on the left (arrows).

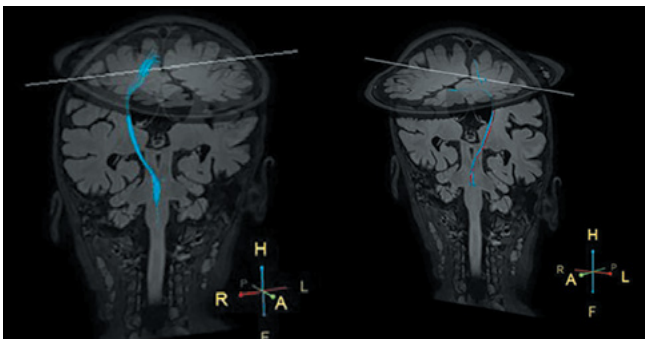


Fig. 2. MR tractogram of patient S. in 6 months from injury. Predominance of CST originating from right SMA (blue).

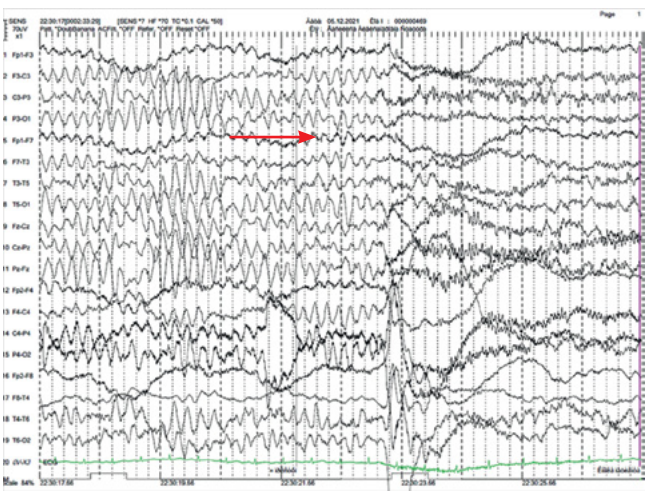
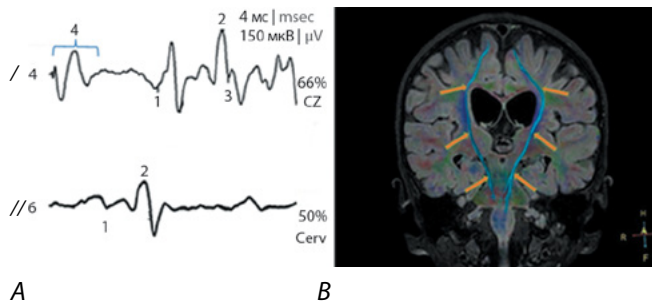


Fig. 3. EEG monitoring in patient S. during the awake stage in 6 months from injury.
Bipolar montage, paper speed 10 sec/page, sensitivity 7 μV/div, low-pass filter: 70 Hz, high-pass filter: 1 Hz. Ictal pattern (red arrow).

During the follow-up 6 months from injury, MEPs could not be reliably verified, although even a single stimulus of 50% intensity triggered a 25-sec generalized tonic seizure. Therefore, the number of stimuli was limited. CST reconstruction on follow-up MR tracto graphy demonstrated substantial volume predominance of the right CST originated from SMA and absence of CST from M1 (Fig. 2).

Video EEG monitoring was performed in external facility. Epileptiform discharges in the vertex region (Fz-Cz-Pz) with periodic spreading to the left central parietal region (C3-P3) or bilaterally were registered in wakefulness and sleep; the prevalence was average in sleep and low in wakefulness. During the recording period, 4 generalized motor tonic seizures accompanied by rhythm desynchronization and fast-wave beta-band activity (ictal pattern) and events of non-epi-



A *B*

Fig. 4. Patient S.'s evaluation 1 year from injury.
A — MEP recording, 66% threshold (*I* — cortical MEP; *II* —segmental MEP). *1* — response isoline deflection point; *2* — maximum isoline positive deflection point; *3* — response reproduction end point; *4* — stimulation artifact.
B — MR tractography: symmetric bilateral CSTs (blue) originating from SMA (arrows).

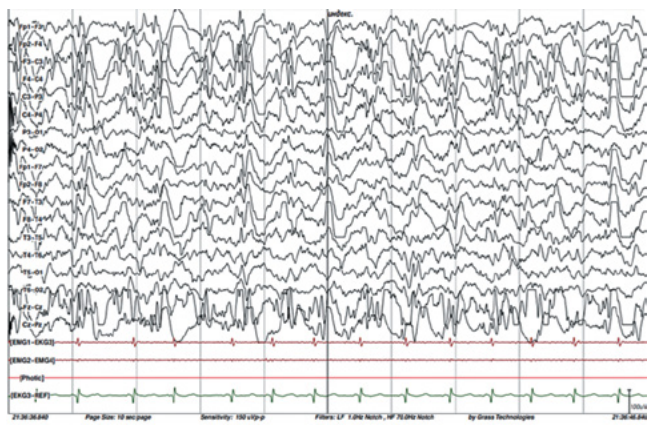


Fig. 5. Sleep EEG in patient S. 1 year from injury.
Bipolar montage, paper speed 10 sec/page, sensitivity 150 μVp-p, low-pass filter: 70 Hz, high-pass filter: 1.0 Hz.

leptologic genesis were recorded (Fig. 3). The child was consulted by an epileptologist, and depakine (8 mg QD, 33 mg/kg QD) and clonazepam (1.5 mg QD) were prescribed.

At 1 year follow-up, reproducible polyphasic contralateral TMS MEP in the left hemisphere was recorded with 66–68% intensity, maximum amplitude of 0.467 mV, and 17.2 msec minimum latency. No response from the left hand muscles during the right hemisphere stimulation was recorded. MR tractography revealed symmetrical CST originating from SMA in the right and left hemispheres with no CSTs from M1 (Fig. 4). No epileptic events were recorded during TMS. The patient received anticonvulsants. Follow-up video EEG monitoring was performed between hospitalizations. No seizure EEG patterns were recorded. In wakefulness and in sleep, regional epileptiform discharges were registered in central-vertex regions (Cz) periodically with spreading to parieto-central regions, mostly bilaterally, as well as independently on the left and right, represented by spike-slow wave complexes, by their morphology the discharges correspond to "rolandic spikes", with high prevalence in some sleep epochs and low prevalence in awake EEG (Fig. 5). The anticonvulsant therapy was adjusted (topiramate 100 mg QD; clonazepam daily dose decreased to 0.625 mg QD).

The fourth assessment was performed in 1.5 years after hypoxic brain injury. MEPs were simultaneously recorded bilaterally on 2 leads from m. abductor pollicis brevis using alternate hemisphere stimulation. No reliable MEP was recorded as response to the stimulation of the right and left hemispheres. The video EEG monitoring findings and the therapy remained unchanged.

Discussion

Studies of TMS diagnostic significance in children with perinatal injuries of the central nervous system (CNS) emphasized clinical, neuroimaging and neurophysiological correlation. Noteworthily, the CST reorganization model depends on the age when the cortical motor areas were injured. In patients under the age of two years, the ipsilateral hemisphere compensates the motor control of the affected limb while excessive neuroplasticity causes neuronal hyperexcitability including, in some cases, epileptiform patterns as a result of hypoxic ischemic encephalopathy [1].

In our observation, massive hypoxic injury in the 3-year-old child led to the activation of CST originated from SMA while the reorganization period was associated with the development of generalized tonic seizures and epileptiform activity that confirms the transient hyperexcitability of cortical neurons. Other motor phenomena were of non-epileptic origin, and they were considered as postanoxic myoclonus manifestations described in the literature as Lance–Adams syndrome [8].

Ipsilateral control of the proximal muscles is described in healthy subjects. In children with early traumatic brain injuries, ipsilateral tracts are primarily involved in motor control of the distal muscles of the limbs [9]. A number of studies demonstrate controversial results regarding M1 excitability depending on the type of reorganization of contra- and ipsilateral tracts in children with perinatal CNS injury [10].

Simultaneous recording of contra- and ipsi-MEPs, similar in latency and shape, including those in children with perinatal stroke and agenesis of the corpus callosum, showed that proprio- and reticulospinal tracts are involved in impulse conduction [9, 11], which challenged the role of commissures in impulse conduction.

SMA tracts, with monosynaptic spinal cord connections, are considered to be less excitable than M1 projections [12]. In our case, massive CNS injury took place at the age of 3 years, during the active development of cortical motor control. This could affect intactness of motor tracts originating from SMA as this area (in addition to the superior parieto-occipital cortex, the anterior intraparietal gyrus, the ventral premotor cortex, the dorsolateral prefrontal cortex, and the posterior and medial parietal gyri) is involved in bimanual movements control, being in fact a part of a multifunctional neural network [13].

Our findings correspond with previously described CST reorganization when SMA represented as an area of hand function motor control in children with brain injuries. However, we have to agree with other researchers and to admit that, due to few clinical observations, we cannot make generalized conclusions about recovery mechanisms in children after acute hypoxic brain injury [14].

In our case, we have observed neuroimaging and neurophysiological dissociation, i.e., the presence of CSTs originated from SMA with no reliably reproducible MEPs in early recovery period after acute hypoxic brain injury. A single registration of contralateral MEP during 1.5-year follow-up cannot be considered as a criterion of recovered motor control.

Conclusion

Our findings indicate the absence of CST recovery after severe hypoxic CNS injury in a 3-year-old child. According to MR-tractography, CST originated from SMA was not clinically associated with recovery of motor function in the child during the described follow-up period. More clinical data is needed to make prognosis on recovery in children after acute hypoxic brain injury based on TMS and MR-tractography.

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