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IMPACT OF 365-DAY ISOLATION ON BIOGENIC AMINE LEVELS AND HEADACHE-RELATED BIOMARKERS: A PROSPECTIVE COHORT STUDY

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Introduction. Preparation for long-duration spaceflights requires assessing the impact of isolation stress on the crew's physiological and psychological status. One of the most common symptoms is headache, driven by biogenic amines and neuropeptides — particularly substance P and calcitonin gene-related peptide (CGRP) — which serve as markers of the body's neuroendocrine response.

Objective. To assess the impact of 365-day isolation in a sealed facility on the levels of biogenic amines and headache markers and to develop effective prevention and correction strategies for cephalgia in crew members during long-duration space missions.

Materials and methods. The SIRIUS-23 isolation experiment was conducted at the Institute of Biomedical Problems of the Russian Academy of Sciences (IMBP RAS) with the participation of 6 individuals (4 females, 2 males; mean age 30.8 ± 4.3 years). Blood and urine sampling was performed during the baseline period (day -7), at multiple isolation time points (days 20, 45, 86, 112, 146, 175, 210, 240, 270, 301, 336, and 364), and during the after-effect period (day +14). High-performance liquid chromatography (HPLC) and enzyme-linked immunosorbent assay (ELISA) were used to evaluate changes in the levels of adrenaline, noradrenaline, dopamine, serotonin, CGRP, substance P, and metabolites (VMA, HVA, and 5-HIAA) in plasma/serum and 24-h urine. Headache intensity and psychoemotional state were assessed using questionnaires and a visual analogue scale (VAS). Statistical analysis was performed using GraphPad Prism 8 software.

Results. Significant variations in catecholamine levels were documented. Adrenaline and noradrenaline levels were significantly higher during the first half of the experiment, with a downward tendency in the second half (starting from day 210). In contrast, metabolite levels (VMA and 5-HIAA) increased in some participants during the second half. Dopamine levels showed no statistically significant differences across isolation phases; however, individual values exceeding the reference range (0–100 pg/mL) were observed (264.18, 118.01, 136.03, and 101.89 pg/mL). On day 146 of the experiment, an increase in serotonin level (reference range: 50–220 ng/mL) was observed in half of the subjects (297.87, 334.84, and 375.93 ng/mL). The values for all subjects on that day were higher than those recorded during the baseline and after-effect periods. Headaches were predominantly episodic and of mild intensity. In the second half of isolation, the frequency and intensity of headache attacks increased, with VAS scores reaching 8–10 points; emotional stress was the primary trigger. Pain exacerbation during physical activity and exposure to sensory stimuli was reported by participants. At the same time, no statistically significant changes or extreme outliers were observed in CGRP and substance P levels when comparing baseline data with measurements obtained during isolation.

Conclusions. One-year isolation altered the metabolism of biogenic amines, reflecting adaptation to stress; however, no significant changes were observed in the levels of specific headache markers (CGRP and substance P). These findings highlight the importance of accounting for environmental factors (temperature, lighting, gas composition, physical activity, and nutrition) when interpreting neurochemical biomarkers in confined environments. Managing these triggers is essential for preventing cephalgia in long-duration isolation experiments and future interplanetary missions.

Keywords: neuro-specific biomarkers; spaceflight; headache; biogenic amines; CGRP; substance P; isolation

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ВЛИЯНИЕ 365-СУТОЧНОЙ ИЗОЛЯЦИИ НА КОНЦЕНТРАЦИЮ БИОГЕННЫХ АМИНОВ И МАРКЕРОВ ГОЛОВНОЙ БОЛИ: ПРОСПЕКТИВНОЕ КОГОРТНОЕ ИССЛЕДОВАНИЕЕ.А. Седов[✉], Г.Ю. Васильева, Д.Ю. Колесников, К.В. Гордиенко, Р.Р. Гимадиев

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Введение. При подготовке к длительным космическим полетам критически важно оценивать влияние изоляции на физическое и психоэмоциональное состояние экипажа. Головная боль — один из самых частых симптомов стресса в замкнутом пространстве, в ее патогенезе ключевую роль играют биогенные амины и нейропептиды (в частности, субстанция Р и пептид, связанный с кальцитонином — CGRP), служащие маркерами нейроэндокринной реакции организма.

Цель. Оценка влияния 365-суточной изоляции в гермообъекте на уровень биогенных аминов и маркеров головной боли для разработки эффективных стратегий профилактики и коррекции цефалгии у членов экипажей длительных космических миссий.

Материалы и методы. В изоляционном эксперименте SIRIUS-23 на базе ГНЦ РФ — ИМБП РАН приняли участие 6 человек (4 женщины, 2 мужчин; средний возраст $30,8 \pm 4,3$ года). Обследование (забор проб крови и мочи) у участников эксперимента SIRIUS-23 проведено в: фоновый период (–7-е сутки), на 20, 45, 86, 112, 146, 175, 210, 240, 270, 301, 336, 364-е сутки и в период последействия (+14-е сутки). Методами высокоэффективной жидкостной хроматографии и иммуноферментного анализа исследовали динамику уровней адреналина, норадреналина, дофамина, серотонина, CGRP, субстанции Р и метаболитов (VMA, HVA, 5-HIAA) в плазме/сыворотке крови и суточной моче. Наличие головной боли и психоэмоциональное состояние оценивали с помощью анкет и визуальной аналоговой шкалы (ВАШ). Статистический анализ проведен с помощью программного обеспечения GraphPad Prism 8.

Результаты. Выявлена значимая динамика катехоламинов: уровни адреналина и норадреналина были достоверно выше в первой половине эксперимента с тенденцией к снижению во второй (начиная с 210-х суток). Концентрация метаболитов (VMA, 5-HIAA) у некоторых участников, напротив, возрастала во второй половине изоляции. Статистически значимых различий в концентрации дофамина у участников эксперимента в различных периодах изоляции выявлено не было, однако были обнаружены значения, выходящие за пределы референтного диапазона (0–100 пг/мл): 264,18; 118,01; 136,03; 101,89 пг/мл. На 146-е сутки эксперимента отмечено увеличение концентрации серотонина (референтный диапазон 50–220 нг/мл) у половины испытуемых: 297,87; 334,84 и 375,93 нг/мл, а значения для всех испытуемых в этот день были выше, нежели в фоновый период и в период последействия. Головные боли носили преимущественно эпизодический характер слабой интенсивности. Однако во второй половине изоляции частота и интенсивность приступов головной боли возросли (до 8–10 баллов), основным триггером являлся эмоциональный стресс. Отмечены жалобы на усиление боли при физической активности и сенсорных стимулах. При этом статистически значимых изменений или экстремальных выбросов в концентрациях нейропептидов CGRP и субстанции Р между фоновыми данными и сессиями изоляции не обнаружено.

Выводы. Годовая изоляция вызывает изменения в метаболизме биогенных аминов, что отражает процессы адаптации к стрессу. Однако достоверных изменений концентрации специфических маркеров головной боли (CGRP и субстанции Р) не выявлено. Полученные данные подчеркивают необходимость контроля факторов среды обитания (температура, освещение, газовый состав, физическая активность, питание) при интерпретации нейробиохимических биомаркеров в замкнутых пространствах, так как мониторинг триггеров является основой профилактики цефалгии в длительных изоляционных экспериментах и будущих межпланетных миссиях.

Ключевые слова: нейроспецифические биомаркеры; космический полет; головная боль; биогенные амины; CGRP; субстанция Р; изоляция

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INTRODUCTION

Headache (cephalalgia) remains one of the most pressing problems in modern medicine, owing to its pronounced interdisciplinary nature. It may reflect not only local pathological processes in the cranial region but also serve as a key clinical symptom of various somatic and psychogenic disorders¹. As early as 1962, a special commission of the National Institute of Neurological Diseases defined cephalalgia as any painful sensation or discomfort projected onto the head area, extending from the superciliary arches to the lower occipital zone [1]. The modern classification presented in the International Classification of Headache Disorders, third edition², divides headaches into three groups: primary cephalalgias (in which no organic cause of pain can be identified), secondary headaches (arising from organic damage to brain structures or other body systems), and cranial neuralgias and facial pain syndromes³. The magnitude of the problem is underscored by epidemiological data. According to the Global Burden of Disease project, cephalalgia consistently ranks among the leading causes of disability worldwide. WHO expert estimates indicate that approximately 40% of the global population is affected⁴.

In the context of manned spaceflight, cephalalgia acquires particular significance. Neuroimaging data from crew members returning from long-duration orbital missions demonstrate significant morphological changes in cerebral structures, including narrowing of the central sulci and subarachnoid spaces and dilation of the ventricular system. These findings are consistent with cranial redistribution of cerebrospinal fluid and suggest possible intracranial hypertension [2–4]. Furthermore, changes in blood biomarkers of brain injury — neuron-specific enolase (NSE) and glial fibrillary acidic protein (GFAP) — have been detected in crew members, indicating possible disruption of the blood-brain barrier [5]. According to a 2024 study of NASA astronauts, 92% of crew members (22 out of 24) reported headaches during spaceflight; approximately 90% were classified as tension-type headaches, and the remaining cases as migraine-like [6]. In the first days of flight, migraine-like pain dominated as part of space motion sickness, whereas in the later period, tension-type headache prevailed. The authors associated this shift with increased intracranial pressure.

However, no significant correlation was found between headache frequency and environmental parameters, including air temperature, oxygen, and carbon dioxide levels. In an earlier pilot study, Vein et al. surveyed 17 astronauts [7]. The findings indicated that 71%

of respondents experienced at least one moderate-to-severe headache episode during their stay in orbit. Specifically, 12% and 18% of participants met the diagnostic criteria for migraine and tension-type headache, respectively; in the remaining 70% of cases, the pathophysiological mechanism could not be identified [7].

Transitioning from normal gravity to weightlessness triggers a cascade of hemodynamic rearrangements and modifies the functional activity of key hormones that regulate circulating fluid volume. These physiological changes represent a compensatory adaptation mechanism to unusual environmental factors and are achieved through the elimination of excess fluid along with electrolytes [8, 9]. At the Institute of Biomedical Problems of the Russian Academy of Sciences, comprehensive studies evaluated water-electrolyte metabolism and associated regulatory systems, specifically the renin-angiotensin-aldosterone axis [10], as well as the glucocorticoid function of the adrenal cortex [11, 12]. The studies involved cosmonauts from long-duration expeditions aboard the Mir orbital station and volunteers in ground-based simulation experiments, including antiorthostatic hypokinesia and dry immersion. The results of these works demonstrated the presence of electrolyte shifts in the blood, hemodynamic changes, and variations in the secretory activity of volume-regulating hormones. These changes may lead to the occurrence of headaches both during the adaptation period to spaceflight and during the readaptation period. To alleviate the condition of cosmonauts during adaptation to microgravity, a set of measures was developed, including the use of mechanical compression devices “Braslet”, application of negative pressure to the lower half of the body, and medicinal agents (furosemide) [13, 14].

Beyond acute adaptation, long-duration spaceflights trigger a complex of “non-psychotic disorders” manifesting as asthenic and functional lability syndromes, which impair operator performance and cause periodic headaches [15]. Moreover, any pain syndromes are accompanied by the development of psychovegetative disorders [16, 17]. Although the physiological responses during acute spaceflight adaptation are well understood, headache remains one of the most common causes of reduced performance in cosmonauts.

In recent years, researchers have focused on neuro-specific proteins — protein S-100, neuron-specific enolase (NSE), and glial fibrillary acidic protein (GFAP) — as markers of brain functional state. Data from dry immersion simulations demonstrate a pronounced correlation between S-100 and NSE blood levels and psychophysiological metrics, including short-term memory capacity and reaction time⁵. In the Mars-105 experiment with six

¹ Loginov VG, Baida AG, Yasinskaya LI. *Headaches: teaching guide*. Minsk: BSMU; 2017 (In Russ.).

² International Classification of Headache Disorders, third edition (ICHD-3; 2018).

³ Headache Classification Committee of the International Headache Society (IHS). The International Classification of Headache Disorders, 3rd ed. *Cephalalgia*. 2013;33(9):629–808. <https://doi.org/10.1177/0333102417738202>

⁴ World Health Organization. Migraine and other headaches: fact sheet. 2025. <https://www.who.int/news-room/fact-sheets/detail/headache-disorders>

⁵ Vasilyeva GYu. Interhormonal interaction and relationship of indicators of neurohumoral and psychophysiological status in humans under simulation of spaceflight conditions: abstract of candidate of medical sciences dissertation. Moscow; 2007 (In Russ.).

healthy volunteers, the levels of S-100, neuron-specific enolase (NSE), and antibodies to myelin-associated glycoprotein (MAG) remained within the reference range, with no deviations in anti-MAG antibodies or corresponding pathological dynamics [18]. However, data from actual spaceflights suggest potential blood-brain barrier disruption under the influence of spaceflight factors [5], consistent with MRI findings showing microstructural remodeling of white matter during isolation [18]. Specifically, the local reduction in fractional anisotropy in the right temporoparietal region is attributed to sensory deprivation. This hypothesis is supported by laboratory analyses showing elevated brain injury biomarkers [5, 18].

Another significant yet understudied component in headache pathogenesis during spaceflights is the functional status of the endothelium. During antiorthostatic hypokinesia, compromised endothelial function coincides with an increase in circulating endothelial cells [19]. Furthermore, post-flight investigations have identified four biological processes — endothelial cell activation, migration, and proliferation, as well as transendothelial migration — that potentially drive spaceflight-induced endothelial dysfunction [20].

An experimental study by the European Space Agency evaluated hematological, endocrinological, and fluid shift parameters in relation to headaches induced by antiorthostatic hypokinesia in 12 healthy male subjects. A total of 26 headache episodes were associated with elevated hematocrit, hemoglobin, and urinary adrenaline levels, as well as with lower relative plasma volumes ($p < 0.05$). During these episodes, endocrinological changes and fluid redistribution were more pronounced, suggesting their role in headache pathophysiology [21].

At the molecular level, a key role in nociceptive signaling in cephalalgia, primarily in migraine, is played by neuropeptides of the trigeminovascular system: substance P and calcitonin gene-related peptide (CGRP). Substance P mediates the transmission of pain afferentation from primary sensory neurons of the trigeminal ganglion to central nociceptive structures, concurrently inducing dilation of meningeal arteries and sustaining neurogenic inflammation [22]. CGRP is one of the most potent endogenous vasodilators: its release from perivascular terminals of trigeminal C-fibers triggers relaxation of cerebral vascular smooth muscle cells and extravasation of plasma proteins into the perivascular space [23]. Plasma CGRP level positively correlates with the intensity of a migraine attack, although in 20–30% of patients its level may not exceed reference values [23]. The recognition of CGRP as a key mediator of the migraine cascade has led to the development of a new class of targeted drugs: monoclonal antibodies to CGRP or its receptor (erenumab, fremanezumab, galcanezumab, and eptinezumab) and small-molecule antagonists (ubrogepant, rimegepant,

and atogepant). These agents have demonstrated high efficacy both in relieving acute attacks and in preventing chronic migraine [24]. Investigating the potential role of CGRP-mediated mechanisms in spaceflight-induced cephalalgia opens the prospect of using anti-CGRP strategies for the pharmacological protection of crew members.

Consequently, understanding the mechanisms of spaceflight-induced headaches requires the integration of data on structural brain changes, hemodynamic shifts, blood-brain barrier integrity, endothelial function, biogenic amines and neuropeptides, and psychophysiological responses under isolation and microgravity conditions. The present study aims to comprehensively evaluate these mechanisms under isolation conditions, with the goal of developing effective prevention and correction strategies for cephalalgia in crew members during long-duration space missions.

The present study aims to assess the impact of 365-day isolation in a sealed facility on the levels of biogenic amines and headache markers, with the goal of informing the development of effective prevention and correction strategies for cephalalgia in crew members during long-duration space missions.

The objectives of the study were:

- quantitative assessment of the frequency and intensity of headache under isolation conditions and during the recovery period;
- determination of the main identifying characteristics and symptoms of headache under isolation conditions and during recovery;
- investigation of the dynamics of neuro-specific biomarkers under isolation conditions and during recovery.

MATERIALS AND METHODS

The study involved two male and four female participants enrolled in the 365-day SIRIUS-23 isolation experiment [25]. At baseline, the participants' characteristics were as follows: mean age, 30.8 ± 4.3 years; height, 168.7 ± 10.6 cm; and weight, 67.2 ± 11.0 kg.

The experiment was conducted at the Institute of Biomedical Problems of the Russian Academy of Sciences (IMBP RAS), in the Ground-Based Experimental Complex (GBEC), where spaceflight conditions — in particular, isolation — are simulated as closely as possible to real space missions.

Participant examinations included venous blood and 24-h urine sampling at baseline (day –7), at multiple isolation time points (days 20, 45, 86, 112, 146, 175, 210, 240, 270, 301, 336, and 364; hereinafter referred to as I20, I45, I86, I112, I146, I175, I210, I240, I270, I301, I336, and I364), and during the after-effect period (day +14; hereinafter I375).

Fasting venous blood samples were collected via venipuncture in the morning with participants in

a supine position; the samples were collected into Acti-Fine tubes (Granat Bio Tech, Russia). The 24-h urine collection began the morning prior to and concluded before blood sampling. Sample preparation adhered to the pre-analytical protocols of the test system manufacturers.

Plasma and serum biogenic amines (adrenaline, noradrenaline, dopamine, and serotonin) and their metabolites (vanillylmandelic acid, VMA; 5-hydroxyindoleacetic acid, 5-HIAA; and homovanillic acid, HVA) in 24-h urine were quantified using high-performance liquid chromatography (HPLC) with amperometric detection on an Agilent 1200 chromatograph (Agilent, USA) equipped with a reversed-phase chromatography column (Phenomenex, USA). Calibration standards and quality control samples were prepared using adrenaline, noradrenaline, dopamine, and serotonin (Merck, USA).

Serum substance P and CGRP levels were evaluated via enzyme-linked immunosorbent assay (ELISA) using biotin-conjugated antibodies with FineTest® kits (China), a Combiwash washer (Germany), and a Humareader HS plate photometer (Germany). Calibration curves were constructed using the standards provided in the kits.

Headaches were assessed using questionnaires [6] that included a visual analogue scale and questions tracking headache frequency, localization, and intensity, as well as outcomes and medication use. Participants completed these questionnaires on the day of venous blood sampling, strictly in the first half of the day after breakfast.

Statistical analysis was performed using GraphPad Prism 8 software. To analyze changes over time, the Friedman test was used. When statistically significant variations were detected ($p < 0,05$), pairwise comparisons were performed via Dunn's post-hoc test, with the Benjamini–Krieger–Yekutieli correction (FDR, $Q = 0,05$) applied for multiple comparisons. Frequencies were compared using Fisher's exact test.

RESULTS AND DISCUSSION

Medical history data and main features of headache episodes among participants

To identify potential triggers for headaches during isolation, detailed interviews were conducted with the subjects to collect anamnestic data. Prior to isolation, headache episodes were highly infrequent in all subjects, occurring from once monthly to once yearly. Headache intensity, assessed via the visual analogue scale, ranged from 3 to 10 points without medication. Subjects could not identify specific triggers, precise localization, or characteristic features of their headaches, while noting no exacerbation with physical activity or stress. These episodes typically resolved spontaneously or with analgesics. Only two

participants experienced severe headache episodes 4 and 12 months prior to isolation; one reported a history of headaches accompanied by visual field disturbances. The participants had not previously consulted physicians for headaches, and no migraine diagnosis had been established for any crew member.

Monthly questionnaire data revealed that, up to day 125 of isolation, the majority of subjects experienced infrequent, low-intensity headaches of unspecified etiology; these episodes either required no treatment or resolved with analgesics. However, one participant reported 6–10 headache episodes prior to I125, with intensities reaching up to 8 points without drug therapy.

After the fourth month of isolation, headache complaints became more frequent. Between days 125 and 175, subjects reported 1–10 episodes, with VAS scores reaching 8 points without medication. These included episodes of unilateral headache exacerbated by physical activity and exposure to stress factors such as light and sound. Furthermore, between days 300 and 363, one participant experienced more than 15 headache episodes. Notably, one of the attacks had a migraine-like character and was unilateral. According to the questionnaire, its intensity rapidly escalated from an initial VAS score of 2–3 to 8 points before being resolved by medication. Another episode manifested as a low-intensity headache (VAS score of 3 points) but was prolonged, lasting up to 10 days.

As the main triggers, the participants identified lack of sleep, uncomfortable neck positioning during sleep, emotional stress, and tension. Headache episodes typically resolved spontaneously (during sleep) or after taking medication.

Following exit from isolation (in the after-effect period), only three crew members reported headache complaints. One of them experienced a continuous, low-intensity headache (VAS score of 2) for up to 10 days post-isolation, with pain exacerbated by stress. Between days 10 and 30 post-isolation, he reported only one episode: a 3-h unilateral headache aggravated by exposure to light or sharp sounds. The other two crew members had low-intensity headaches during this period, lasting 6–12 h, which were similarly exacerbated by physical activity and exposure to stressors.

The obtained data are consistent with the results of studies in real spaceflight conditions. According to van Oosterhout et al., 92% of NASA astronauts (22 out of 24) reported headaches during spaceflight, with the majority being of tension-type headache (~ 90%), and the remaining cases being migraine-like [6]. Previously, Vein et al. showed that 71% of astronauts experienced at least one moderate-to-severe headache episode in orbit, though the underlying pathophysiological mechanism could not be identified in 70% of cases [7]. The predominance of stress-induced, tension-type cephalalgia in our cohort corroborates these observations,

confirming that tension-type headache is the predominant form of cephalgia during long-term isolation. The increase in headache frequency and intensity in the second half of the experiment may be due to the cumulative effect of psychoemotional stress and sleep disturbances, which was previously noted in the context of “non-psychotic disorders” with the development of asthenic syndrome during long-duration spaceflights [15].

Changes in biogenic amine and metabolite levels during the SIRIUS-23 isolation experiment

Plasma adrenaline levels across the subjects remained within the reference range (0–140 pg/mL). Although no significant differences were found between the experimental time points overall ($p > 0.05$, Fig. 1), higher concentrations were noted during the first half of the experiment, up to day 175 (Fisher’s exact test, $p < 0.05$; Fig. 1). Specifically, three subjects exhibited elevated adrenaline levels at baseline and on day 20 of isolation. In one individual, these values persisted up to day 113 (baseline: 87.438 pg/mL; I20: 95.576 pg/mL; I45: 79.15 pg/mL; I86: 55.744 pg/mL; I113: 73.887 pg/mL), before dropping to minimum values at points I275, I337, and I363 (17.393, 19.757, and 20.085 pg/mL). The lowest concentrations recorded throughout the entire observation period (10.262 and 11.852 pg/mL) were noted on day 240.

During the study, statistically significant variations in noradrenaline levels occurred between baseline and specific isolation time points ($p < 0.05$; Fig. 2). In the second half of the experiment, (starting from day 210), noradrenaline level was lower than in the first half (up to day 175). No noradrenaline concentrations significantly exceeded reference or baseline levels. The highest

recorded levels occurred in a single subject: 825.18 pg/mL at baseline, 795.96 pg/mL at I86, 695.08 pg/mL at I45, and 620.12 pg/mL 13 days later, during the after-effect period.

No statistically significant differences in dopamine levels were found among the participants across the different periods of isolation (Fig. 3). However, elevated dopamine values exceeding the reference range (0–100 pg/mL) were detected in two subjects. Specifically, in one participant during the second half of the experiment, the dopamine level reached 264.18 pg/mL on day 240 and 118.01 pg/mL on day 363; in the second participant, elevated values were recorded in the first half of isolation on day 45 (136.03 pg/mL) and day 86 (101.89 pg/mL).

While the mean serotonin levels for the cohort remained within the physiological norm (50–220 ng/mL; Fig. 4), individual values exceeding the upper and lower reference limits were observed. In one crew member, serum serotonin levels were 317.19 ng/mL on day 20, 297.87 ng/mL at I145, 334.84 ng/mL at I275, and 375.93 ng/mL at I363. Notably, at point I146, an increase in serotonin levels was noted in half of the subjects; the absolute values across all subjects on that day exceeded both baseline and after-effect values.

Analysis of biogenic amine metabolites in urine demonstrated that VMA levels in most participants fell within the reference range (1.400–6.500 mg/day). No statistically significant differences in serotonin levels were found across the study periods (Fig. 5).

Analysis of VMA levels revealed eight values exceeding the reference range, five of which belonged to one subject (12.4, 8.68, 7.95, 7.46, and 7.24 mg/day). All

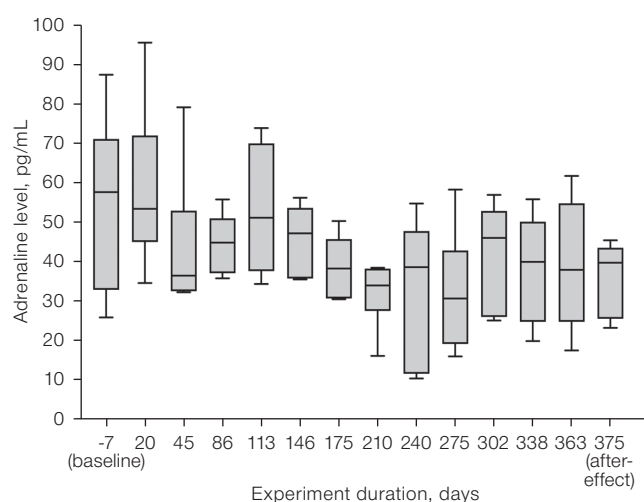


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Fig. 1. Median plasma adrenaline levels in the SIRIUS-23 crew during various isolation phases

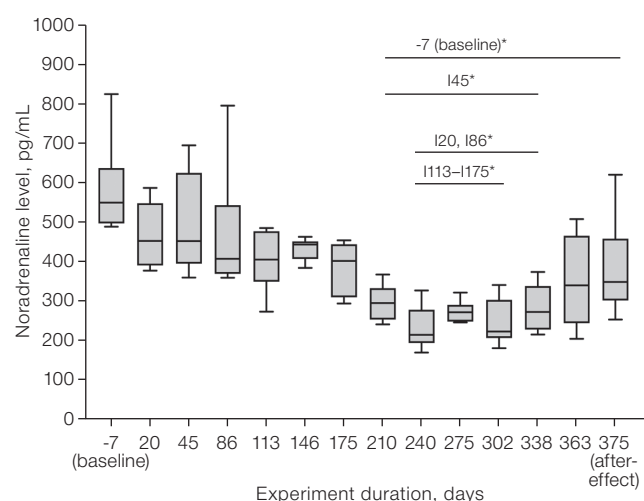


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Fig. 2. Median plasma norepinephrine levels in the SIRIUS-23 crew during various isolation phases.

* — horizontal lines above the graph indicate periods (days) for which statistically significant differences were found compared to the points indicated above the respective line ($p < 0.05$)

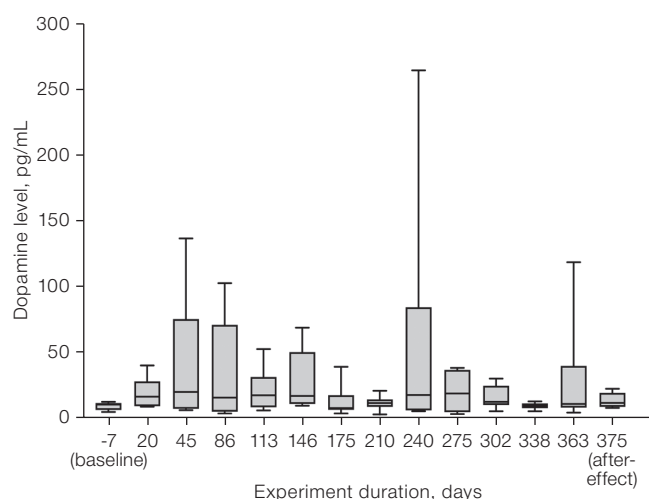


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Fig. 3. Median plasma dopamine levels in the SIRIUS-23 crew during various isolation phases

cases of exceeding the upper reference limit were detected in the second half of the experiment (I210, I240, I275, I335, and I363).

However, despite stable central tendencies, distribution analysis (Fig. 5) showed an increase in interindividual variability during the second half of isolation. To quantify this effect, we analyzed the temporal variations in pairwise absolute differences between all subjects (15 pairs per time point). Comparison of these datasets at different isolation time points revealed statistically significant differences in the degree of intragroup variability across the course of the experiment ($p < 0.05$).

The smallest spread of values — representing the greatest group homogeneity — was observed at points I45, I86, I113, and I175. Starting at I240, a statistically significant increase in variability compared to these time points emerged ($p < 0.05$ for all comparisons) and persisted until the end of the experiment. Time point I210 captured a transitional phase: although differences became more pronounced, they did not reach statistical significance across all pairwise comparisons with the baseline time points (I45 and I175).

The concentration of 5-HIAA in 24-h urine (Fig. 6) exceeded the reference range (2–7 mg/day) in some participants. For instance, one subject demonstrated an extremely elevated 5-HIAA value of 23.542 mg/day at I240, with a downward trend to 10.739 mg/day by I275. Another subject showed elevated levels of 13.467 and 13.085 mg/day, respectively, during the same observation intervals. Notably, despite the absence of significant cohort-wide changes, a marked tendency toward elevated 5-HIAA levels emerged in the majority of crew members starting from day 145.

No extreme or markedly deviating HVA levels were recorded in the 24-h urine of participants relative to the rest of the cohort (Fig. 7), except for a single spike to

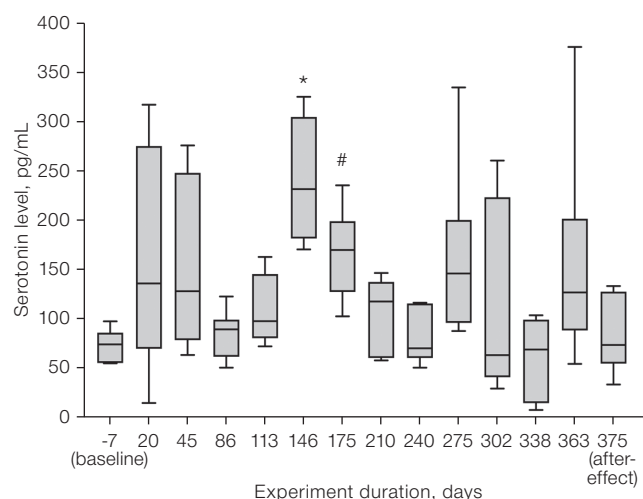


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Fig. 4. Mean serum serotonin levels in the SIRIUS-23 crew during various isolation phases. * — indicates a statistically significant difference ($p < 0.05$) compared to baseline, I86, I240, I302, I338, and I375; # indicates $p < 0.05$ compared to baseline and I338

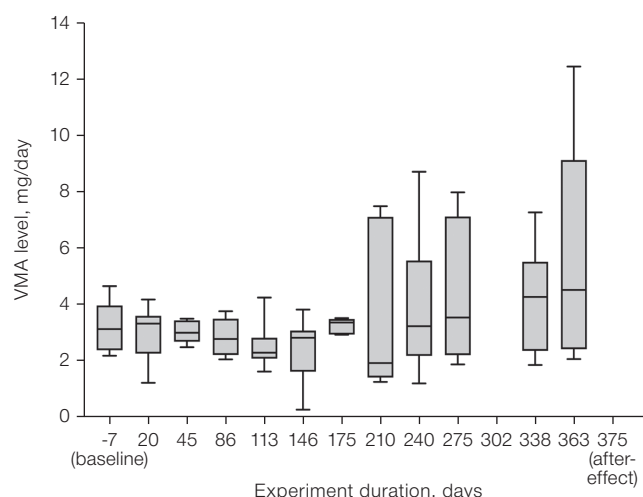


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Fig. 5. Median VMA levels in 24-h urine samples from the SIRIUS-23 crew during various isolation phases

9.42 mg/day documented in one subject on day 20. However, analysis revealed significant differences in HVA concentrations among participants during different isolation periods. Specifically, HVA levels were significantly lower at point I210 relative to baseline (day -7), as well as compared to values at points I20, I45, and I175.

Under one-year isolation conditions, the sympathoadrenal system exhibited a distinct biphasic response: elevated levels of adrenaline and noradrenaline were observed in the first half of the experiment (up to day 175), followed by a significant decrease during the second half (after day 210). Dopamine metabolism,

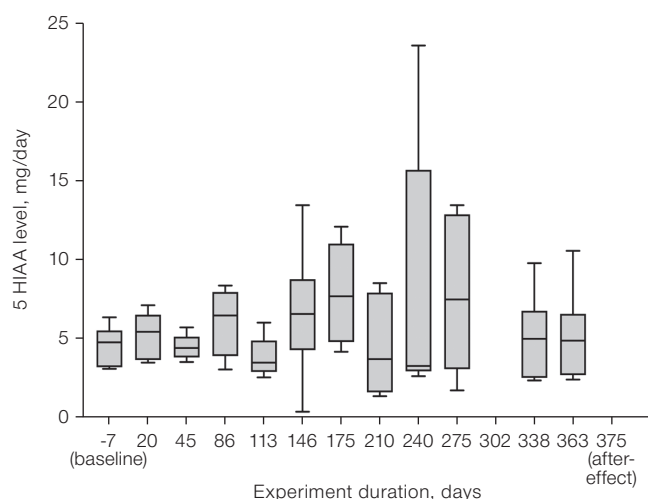


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Fig. 6. Median 5-HIAA levels in 24-h urine samples from the SIRIUS-23 crew during various isolation phases

assessed by HVA excretion, was statistically significantly reduced at point I210 compared to early time points (baseline, I20, I45, and I175). Concurrently, urinary 5-HIAA levels showed an upward tendency in the majority of subjects starting from day 145, despite the absence of a cohort-wide statistically significant difference from baseline. Individual variability of the analyzed variables increased toward the end of isolation, with occasional spikes in dopamine and serotonin observed in some participants.

These findings align with published data regarding the role of catecholamine and serotonin metabolism disturbances in headache pathogenesis. D'Andrea et al. showed that in episodic migraine without aura, plasma profiles exhibit elevated dopamine and reduced noradrenaline levels, driven by a metabolic shift toward increased tyrosine decarboxylase activity with a concurrent decrease in hydroxylase activity [26]. This dopamine system dysfunction is associated with the development of migraine prodromal symptoms [27], whereas altered functional connectivity between the nucleus accumbens and the amygdala — modulated by D2/D3 receptors — may underlie emotional distress and pain intensity [28]. In contrast, noradrenaline performs an antinociceptive function: the locus coeruleus modulates nociceptive signal transmission through projections to the thalamus and the dorsal horns of the spinal cord, meaning its reduction weakens the descending inhibitory influence on pain pathways [29, 30]. Serotonin also plays a central role: a meta-analysis by Frederiksen et al. revealed significantly higher serotonin levels in episodic migraine with aura, whereas chronic migraine and chronic tension-type headache were associated with depletion of the serotonin system [31, 32]. Finally, the elevated 5-HIAA excretion in the second half of our experiment

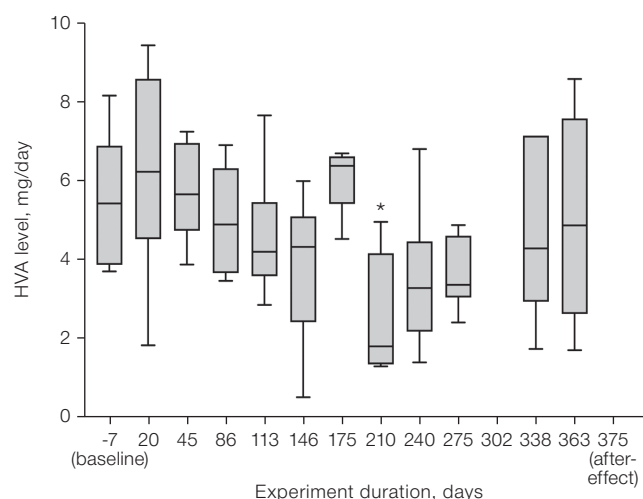


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Fig. 7. Median HVA levels in 24-hour urine samples from the SIRIUS-23 crew during various isolation phases. * — indicates a statistically significant difference ($p < 0.05$) compared to baseline, I20, I45, and I175

corroborates data identifying increased serotonin metabolism as a characteristic response to chronic stress [33].

The decline in noradrenaline, combined with elevated serotonin metabolites and episodic dopamine spikes during the second half of isolation, creates a neurochemical profile that may contribute to the higher headache frequency and intensity observed in some crew members during this period. However, while the overall neurochemical findings align with pathophysiological models of cephalalgia, the available data do not permit establishing direct causal relationships between specific biogenic amine fluctuations and headache episodes.

Longitudinal variations in neuro-specific protein levels in SIRIUS-23 participants

Substance P and CGRP are key effectors of the trigemino-vascular system. Their joint release from sensory endings innervating the meninges initiates a cascade of neurogenic inflammation — vasodilation, plasma extravasation, mast cell degranulation, and sensitization of pain pathways [34, 35]. While CGRP stands out as the most potent cerebral vasodilator and the main effector of the migraine attack — a role that has driven the development of targeted anti-CGRP drugs [24, 36, 37], substance P predominantly mediates plasma extravasation and neurogenic edema [22, 38]. Notably, the feasibility of using CGRP as a biomarker has been confirmed in studies of pain syndromes in military personnel [39].

During the isolation period, CGRP and substance P concentrations demonstrated no statistically significant intragroup variations across the different time points (Figs. 8, 9). The absence of statistically significant

changes in CGRP and substance P in our experiment may indicate that, despite the increase in headache frequency in the second half of isolation, the trigeminovascular system was not activated to a degree sufficient for the formation of a migraine attack. This is consistent with the predominantly tension-type nature of the cephalalgia identified from the questionnaire data. Nevertheless, the pronounced individual variability in neuropeptide dynamics indicates the need for a personalized approach to monitoring these biomarkers.

It should be noted that prolonged human isolation in sealed facilities with an artificial habitat is associated with exposure to a heterogeneous set of factors — including temperature fluctuations, changes in photoperiod and spectral composition of lighting, deviations in the partial pressure of oxygen and carbon dioxide, hypodynamia, a specific physical activity regimen, as well as particularities of nutritional support and, indirectly, the state of the gut microbiota. Each of these factors is capable of independently modulating the metabolism of biogenic amines (dopamine, norepinephrine, epinephrine, serotonin) and the levels of neuropeptides (CGRP, substance P) — key effectors of the trigeminovascular system and neurogenic inflammation, which underlie the pathophysiology of primary headaches.

Consequently, when interpreting changes in the concentration of these mediators in plasma, urine, or cerebrospinal fluid in individuals under long-term isolation conditions, correction for the combined effect of the above factors is required. The obtained data emphasize the mandatory multifactorial monitoring of the living environment and standardization of experimental conditions in studies aimed at identifying the pathophysiological mechanisms of cephalalgia under isolation, as well as in the development of personalized strategies for the prevention and therapy of headache in enclosed spaces. In the next stages of the work, we plan to conduct

an analysis of isolation factors with an assessment of the contribution of each of them to the development of headache.

The obtained results allow us to formulate promising directions for further study of adaptation mechanisms in isolation. First of all, it seems justified to abandon a purely symptomatic approach to headache treatment (use of analgesics post factum) in favor of preventive neurometabolic correction. Given the identified heterogeneity of responses (high interindividual variability of catecholamine metabolites and neuropeptides), such a strategy requires personalized monitoring of the state of each crew member. Longitudinal monitoring of biogenic amines and neuropeptides opens opportunities for personalized, predictive medicine in extreme environments. The study of biogenic amine and neuropeptide levels in individuals under long-term isolation may have not only fundamental but also applied significance. Detection in a specific individual of an initially high level of CGRP or an imbalance in the catecholamine system may indicate a predisposition to the development of headache under stressful conditions and serve as a basis for including such a subject in a risk group, followed by the development of personalized preventive measures. In addition, longitudinal determination of these biomarkers allows assessment of the effectiveness of preventive interventions (pharmacological, physical, psychocorrective).

The practical implementation of this approach is seen in a comprehensive impact on different links of pathogenesis: creating conditions for reducing chronic cortisol-mediated stress; enriching the diet with dietary precursors of dopamine and noradrenaline (L-tyrosine) and coenzymes for neurotransmitter synthesis (B vitamins); introducing short-term sessions of high-intensity physical training; regulating circadian rhythms to maintain dopaminergic tone in the absence of natural sunlight using phototherapy methods.

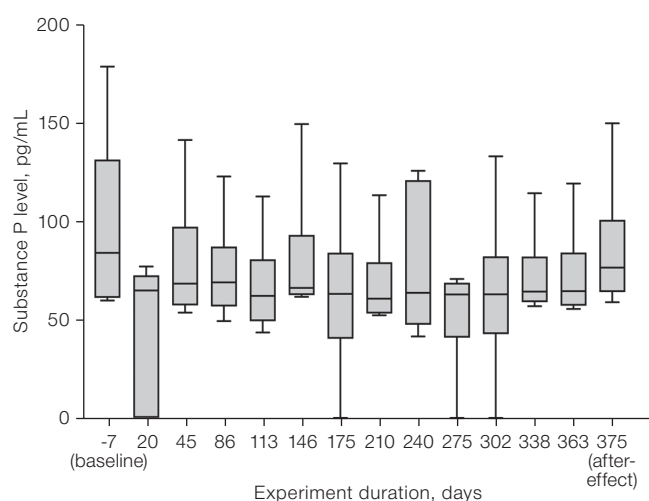


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Fig. 8. Median serum substance P levels in the SIRIUS-23 crew during various isolation phases

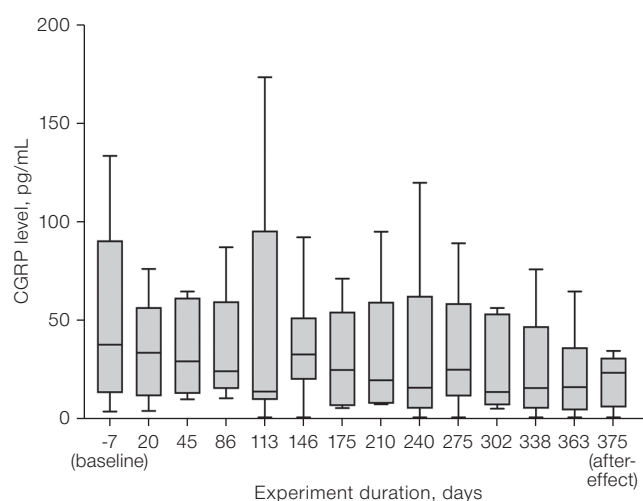


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Fig. 9. Median serum CGRP levels in the SIRIUS-23 crew during various isolation phases

CONCLUSIONS

1. Under conditions of one-year isolation, headache was episodic, of mild or moderate intensity. All recorded headache episodes had a favorable outcome, resolving spontaneously or with standard analgesics. This confirms the reversible, functional nature of the identified disturbances and the absence of organic brain damage. However, a statistically significant increase in headache episodes in some crew members, which reached moderate to high intensity (up to 8–10 points) in the second half of isolation, correlates with signs of neurotransmitter system depletion.
2. The significant decrease in adrenaline and noradrenaline levels during the second half of the isolation experiment may indicate a gradual depletion of the sympathoadrenal system under chronic stress, without a preceding phase of sustained hyperactivation.
3. The statistically significant decrease in HVA excretion on day 210 of isolation compared to baseline and early time points is a key marker of latent depletion of the do-

paminergic system in response to the prolonged stress factor of isolation. The observed significant increase in individual variability of all studied parameters (especially VMA, 5-HIAA) toward the end of the isolation period, along with episodic spikes in dopamine and serotonin in some participants, may indicate heterogeneity of adaptation strategies and the need for personalized monitoring. The pronounced tendency toward an increase in 5-HIAA excretion in the majority of subjects, starting from day 145 of isolation, is consistent with literature data on increased serotonin metabolism as a characteristic response to chronic stress. These neurochemical shifts in the second half of the experiment are associated with a deterioration in well-being and an increase in the frequency and intensity of headache in some subjects.

4. No statistically significant differences were found in CGRP and substance P concentrations across the various phases of isolation. Nevertheless, the pronounced individual variations in both markers may indicate a high interindividual variability of the neuropeptide response under long-term isolation.

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