

Byshek M.Yu., Berezovskyi A.M., Makoveiev D.S.¹, Solyeyko O.V.¹, Chernykh M.O., Mironov Ye.V.
National Pirogov Memorial Medical University, Vinnytsya, Ukraine;
¹Kyiv Medical University, Kyiv, Ukraine

MORPHOLOGICAL CHANGES IN THE BRAIN AND ADRENAL GLANDS UNDER CHRONIC IMMOBILIZATION STRESS AND THEIR CORRECTION WITH MELATONIN

e-mail: berzovskiy@gmail.com

The purpose of the study was to determine morphological changes in the brains and adrenal glands of rats under chronic immobilization stress and to evaluate these changes under melatonin administration. Fifteen sexually mature male Wistar rats were divided into three groups (n=5): control, chronic immobilization stress, and chronic immobilization stress with melatonin (5 mg/kg orally for 21 days). Serum cortisol concentration was measured by immunochemiluminescence; brain and adrenal samples were examined after hematoxylin and eosin staining. Immobilization stress was accompanied by an increase in cortisol levels from 1.66 ± 0.20 to 13.80 ± 0.33 nmol/L ($p < 0.05$), vascular engorgement, erythrocyte stasis or aggregation, and expansion of perivascular and pericellular spaces in the brain. In the melatonin group, cortisol levels were 11.04 ± 0.28 nmol/L, 20% lower than in the uncorrected stress group ($p < 0.05$); the microcirculatory and tissue changes described were less pronounced. The adrenal architecture remained intact; however, vascular and cytoplasmic changes differed between groups. The data indicate a partial protective effect of melatonin under chronic immobilization stress; confirmation requires quantitative morphometry, corticosterone measurement, and molecular markers of neuroinflammation and autophagy.

Key words: chronic immobilization stress, melatonin, brain, adrenal glands, morphology, neuroprotection.

Бишек М.Ю., Березовський А.М., Маковець Д.С., Солейко О.В., Черних М.О., Міронов Є.В. МОРФОЛОГІЧНІ ЗМІНИ ГОЛОВНОГО МОЗКУ ТА НАДНИРКОВИХ ЗАЛОЗ ЗА УМОВ ХРОНІЧНОГО ІММОБІЛІЗАЦІЙНОГО СТРЕСУ ТА ЇХ КОРЕКЦІЯ МЕЛАТОНІНОМ

Метою дослідження було охарактеризувати морфологічні зміни головного мозку та надниркових залоз щурів за умов хронічного іммобілізаційного стресу та оцінити їх зміни під впливом мелатоніну. П'ятнадцять статевозрілих щурів-самців лінії Wistar розподілили на три групи (n=5): контроль, хронічний іммобілізаційний стрес та хронічний іммобілізаційний стрес з мелатоніном (5 мг/кг перорально протягом 21 доби). Концентрацію кортизолу в сироватці крові визначали методом імунохемілюмінесценції; зразки мозку та надниркових залоз досліджували після забарвлення гематоксиліном та еозином. Іммобілізаційний стрес супроводжувався зростанням рівня кортизолу з $1,66 \pm 0,20$ до $13,80 \pm 0,33$ нмоль/л ($p < 0,05$), повнокрів'ям судин, стазом або агрегацією еритроцитів і розширенням периваскулярних та перицелюлярних просторів у головному мозку. У групі мелатоніну рівень кортизолу становив $11,04 \pm 0,28$ нмоль/л, що на 20% нижче, ніж у групі стресу без корекції ($p < 0,05$); описані мікроциркуляторні та тканинні зміни були менш вираженими. Архітектура надниркових залоз залишалася збереженою, однак судинні та цитоплазматичні зміни відрізнялися між групами. Отримані дані свідчать про частковий протекторний ефект мелатоніну за умов хронічного іммобілізаційного стресу; для підтвердження потрібні кількісна морфометрія, визначення кортикостерону та молекулярні маркери нейрозапалення й аутофагії.

Ключові слова: хронічний іммобілізаційний стрес, мелатонін, головний мозок, надниркові залози, морфологія, нейропротекція.

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Chronic stress is accompanied by long-term neuroendocrine and metabolic shifts that can contribute to damage to the nervous and endocrine systems [1, 11]. A key link in the stress response is activation of the hypothalamic-pituitary-adrenal axis, which increases glucocorticoid secretion and modulates the immune response [4]. In rats, the main glucocorticoid is corticosterone; in humans, it is cortisol. This interspecies difference must be considered when interpreting experimental data.

In the central nervous system, a prolonged stress response is associated with microcirculatory and vasomotor dysregulation [8], oxidative stress, neuroinflammatory responses, and changes in

neuroplasticity. At the same time, chronic immobilization is a model of stress, not a full experimental equivalent of clinical post-traumatic stress disorder, which requires verification of a complex of behavioral and neurobiological signs [12].

Melatonin regulates circadian rhythms and also exhibits antioxidant and anti-inflammatory properties [3, 7, 10, 13, 14]. In experimental models of chronic immobilization stress, it reduced the severity of the inflammatory response, apoptosis, and pyroptosis of microglia in the hippocampus [6, 15]. However, the morphological changes in the brain and adrenal glands under these conditions and the possibility of

their correction by melatonin require further comparison.

The purpose of the study was to determine morphological changes in the brains and adrenal glands of rats under chronic immobilization stress and to evaluate these changes under melatonin administration.

Materials and methods. The study was conducted on 15 sexually mature white male Wistar rats weighing 200–250 g. Animals had free access to water and food except during periods of immobilization. The room temperature was 19–23 °C, and the relative humidity was 50–75%.

Rats were randomly divided into three groups (n=5). Animals in Group 1 (control) were not immobilized and received an equal volume of 1% starch gel. In group 2, chronic stress was simulated by daily 12-hour immobilization (21:00–09:00) with a 12-hour recovery period for 21 days [5]. Animals in group 3 were similarly exposed and received daily oral melatonin at a dose of 5 mg/kg in 1% starch gel. Immobilization was performed using a splint with individual compartments.

Serum cortisol concentration was determined by the Chemiluminescence immunoassay method on an ACCESS 2 analyzer (Beckman Coulter, USA). Blood was obtained after euthanasia of the animals by sevoflurane overdose followed by decapitation. Serum was obtained by centrifugation at 1200 g for 15 min at 19–23 °C; aliquots were stored at –20 °C.

For morphological examination, brain and adrenal gland samples were fixed in 10% neutral buffered formalin (pH 7.0) for 48 h. Paraffin sections were stained with hematoxylin and eosin [2] and examined using an Olympus CX23 microscope with a digital camera.

Results are presented as mean $\pm$ standard error

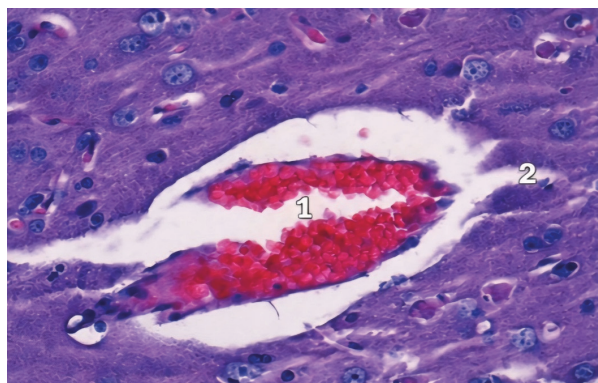


Fig. 1. Brain of a rat of group 2: 1 – aggregation of erythrocytes in the lumen of the vessel; 2 – expansion of the perivascular space. Hematoxylin and eosin staining. $\times 400$.

The cortex's cytoarchitecture was preserved, with six layers clearly defined. Most neurons were oval or triangular, with distinct nuclei and nucleoli. No pronounced cellular inflammatory infiltration or sclerotic changes were detected in the examined sections.

of the mean ($M \pm m$). Intergroup differences were assessed using Student's t-test in Statistica 6.0 (StatSoft Inc., USA); differences were considered statistically significant at $p < 0.05$.

Animal procedures were performed in accordance with the European Convention for the Protection of Vertebrate Animals used for Research and Other Scientific Purposes (Strasbourg, 1986) and Directive 2010/63/EU. Minutes of the local bioethics commission: No. 9 dated 03/19/2025.

Results of the study. The concentration of cortisol in the serum of control animals was 1.66 ± 0.20 nmol/L. In the chronic immobilization stress group, it increased to 13.80 ± 0.33 nmol/L ($p < 0.05$ compared to control). Under melatonin use, the indicator was 11.04 ± 0.28 nmol/L, 20% lower than in the stress group without correction ($p < 0.05$).

In rats in group 2, the pia mater had normal thickness; no cellular inflammatory infiltration was detected. The vessels of the membranes and brain substance were engorged with blood; stasis and erythrocyte aggregation were observed in some vessels. The perivascular and pericellular spaces were dilated (Fig. 1).

The cytoarchitecture of the cortex remained preserved. In some fields of view, neurons had clear contours, normochromic nuclei, and nucleoli; in others, neurons with basophilic cytoplasm and altered shape were detected. No signs of sclerosis were detected. Hematoxylin and eosin staining cannot reliably determine astrocyte or microglial activation.

In group 3, the pia mater had normal thickness without cellular infiltration. Blood filling was moderate in most vessels; in some areas, vessels with narrow lumens were identified. The perivascular and pericellular spaces remained somewhat dilated (Fig. 2).

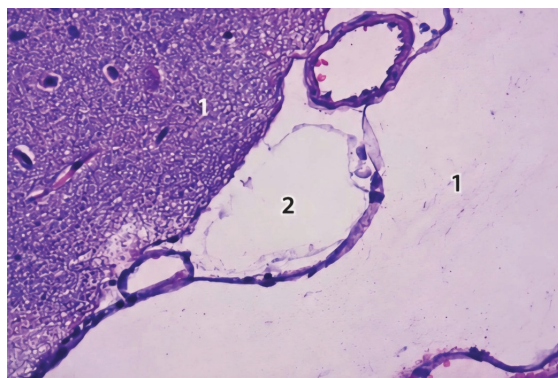


Fig. 2. Brain of a rat of group 3: 1 – pia mater; 2 – vessel with a narrow lumen. Hematoxylin and eosin staining. $\times 400$.

In the adrenal glands of group 2 animals, the cortical and medullary substances were clearly demarcated. Glomerular, fascicular, and reticular zones were identified in the cortex; endocrinocytes had clear contours and stained nuclei. Most capillaries were full-blooded. In the vessels of the

capsule and medulla, stasis or separation of plasma from the formed elements was detected in some areas (Fig. 3).

In group 3, the structure and ratio of the adrenal gland layers were preserved. Endocrinocytes and

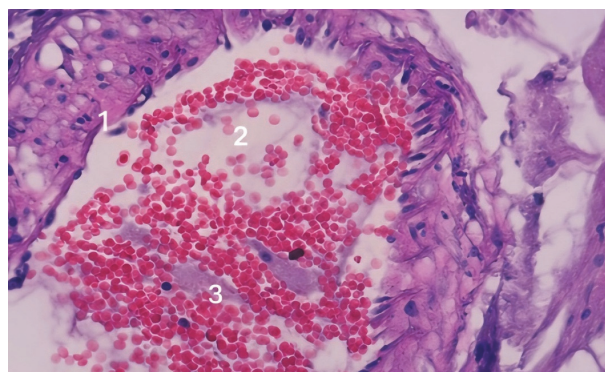


Fig. 3. Adrenal gland capsule vessel of group 2 rats: 1 – wall delamination; 2 – separation of plasma and formed elements; 3 – leukocytes in the lumen. Hematoxylin and eosin staining. $\times 400$.

Discussion. Chronic immobilization was accompanied by a marked increase in measured cortisol concentrations, consistent with activation of the hypothalamic-pituitary-adrenal axis under conditions of prolonged stress [1, 11]. Melatonin reduced this indicator, but did not normalize it. At the same time, in rats, the main circulating glucocorticoid is corticosterone, so cortisol should be considered an additional marker. Validating the test system for rat serum and determining corticosterone in parallel would strengthen the evidence base for the results.

Vascular congestion, erythrocyte stasis or aggregation, and expansion of perivascular and pericellular spaces in the brain indicate impaired microcirculation and water metabolism. The lower severity of these changes under melatonin administration is consistent with experimental data showing its ability to attenuate the inflammatory response, apoptosis, and pyroptosis in microglia in models of chronic immobilization stress [6, 15]. However, our study did not detect GFAP, Iba1, NLRP3, or other markers, so we cannot directly prove

their nuclei had clear contours. Intracytoplasmic pigment inclusions were detected in some cells of the fascicular zone (Fig. 4). Hematoxylin and eosin staining cannot reliably identify them as lipofuscin or as a marker of autophagy.

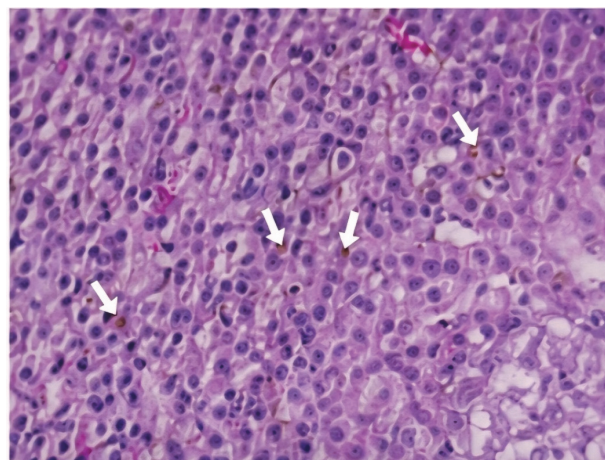


Fig. 4. The fascicular zone of the adrenal cortex of a group 3 rat; arrows indicate intracytoplasmic pigment inclusions. Hematoxylin and eosin staining. $\times 400$.

an anti-inflammatory mechanism based on hematoxylin and eosin staining data.

Preservation of overall adrenal architecture with concomitant vascular changes may reflect the organ's functional adaptation to prolonged stress; melatonin's protective effects on the adrenal glands have been described in other experimental models [9]. Pigment inclusions in the zona fasciculata require confirmation by special histochemical methods or autofluorescence. To assess autophagy, it is advisable to measure LC3B, p62, and other validated markers; hematoxylin and eosin staining alone does not provide sufficient evidence to conclude that this process is activated.

The main limitations of the work are the small number of groups ($n=5$), the lack of quantitative morphometry and blinded drug assessment, the measurement of only cortisol rather than corticosterone, and the lack of immunohistochemical or molecular verification of the described processes. These limitations do not provide grounds for direct extrapolation of the obtained data to clinical PTSD.

Conclusions

1. Chronic immobilization of rats was accompanied by an increase in serum cortisol concentration to 13.80 ± 0.33 nmol/L compared to 1.66 ± 0.20 nmol/L in the control ($p < 0.05$).
2. In the brains of animals in the stress group, vascular congestion, stasis or aggregation of erythrocytes, and expansion of perivascular and pericellular spaces were detected. At the same time, the cortex's general cytoarchitecture was preserved.
3. The use of melatonin reduced the cortisol concentration to 11.04 ± 0.28 nmol/L, which is 20% lower than the indicator in the stress group without correction ($p < 0.05$). This was accompanied by less severe microcirculatory and tissue changes.
4. The obtained data support the hypothesis of a partial protective effect of melatonin under conditions of chronic immobilization stress, but require confirmation by quantitative morphometry, determination of corticosterone, and specific molecular markers.

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Conflict of interest. The authors have no conflicts of interest to declare.

ORCID: Byshek M.Yu. <https://orcid.org/0009-0006-2092-7294>, Berezovskyi A.M. <https://orcid.org/0000-0001-8791-6713>, Makoveiev D.S. <https://orcid.org/0009-0007-8761-998X>, Solyeyko O.V. <https://orcid.org/0000-0002-7233-2886>, Chernykh M.O. <https://orcid.org/0000-0002-4093-5957>, Mironov Ye.V. <https://orcid.org/0000-0003-0530-484X>.

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