

Quantitative changes in monoamines in visual brain structures (visual cortex, colliculus superior, and lateral geniculate dorsales) in an experimental model of retinal pigmentary dystrophy

Khuraman Miryusifova ^{1,2}, Arzu Allahverdiyeva ¹, Nigar Huseynova ¹, Gunay Malikova ¹, Kamala Cavadova ¹, Alipanah Huseynov ¹, Gulsum Abdullayeva ¹, Sabina Shahmaliyeva ¹, Gunel Valiyeva ¹

¹ Living Systems Research Institute Public Legal Entity, Ministry of Science and Education, Baku (Republic of Azerbaijan)

² Baku State University, Baku (Republic of Azerbaijan)

Кількісні зміни моноамінів у зорових структурах мозку (зорова кора, верхній горбик та латеральні колінчасті дорсальні ядра) в експериментальній моделі пігментної дистрофії сітківки

Хураман Мірюсіфова ^{1,2}, Арзу Аллахвердієва ¹, Нігар Гусейнова ¹, Гюнай Малікова ¹, Камала Джавадова ¹, Аліпана Гусейнов ¹, Гюльсум Абдуллаєва ¹, Сабіна Шахмалієва ¹, Гюнель Валієва ¹

¹ Публічна юридична особа «Інститут досліджень живих систем», Міністерство науки та освіти, Баку (Азербайджанська Республіка)

² Бакинський державний університет, Баку (Азербайджанська Республіка)

Abstract

Introduction. Retinal pigment dystrophy is characterized not only by degeneration of peripheral photoreceptors but also by disruption of neuromodulatory balance in the central structures of the visual analyzer.

Purpose. The specific aim of the study was to determine quantitative changes in serotonin (5-HT), norepinephrine (NA), and dopamine (DA) levels in the visual cortex (VC), superior colliculus (CS), and lateral geniculate dorsales

(LGD) under experimentally induced retinal pigment dystrophy (RPD).

Methods. The study was conducted on rabbits, where a dystrophy model was induced by injection of monoiodoacetic acid. The concentration of monoamines in brain structures was determined using a fluorometric analysis method.

Results. Under normal conditions, 5-HT had the highest value (410 ± 7 ng/g in VC, 380 ± 10 ng/g in CS, 405 ± 5 ng/g in LGD). A noticeable increase in 5-HT (420 ± 10 ng/g) and DA (390 ± 10 ng/g) was observed in the CS, while a decrease in 5-HT (365 ± 5 ng/g) and NA (320 ± 10 ng/g) was recorded in the VC. In the LGD, dopamine levels decreased significantly to 310 ± 6 ng/g ($p < 0.05$).

Under experimental retinal pigment dystrophy, significant and structure-specific alterations in monoamine levels were observed in the central visual structures. In the CS, the levels of 5-HT, NA, and DA increased from 380 ± 10 to 420 ± 10 ng/g, from 325 ± 7 to 360 ± 10 ng/g, and from 335 ± 6 to 390 ± 10 ng/g, respectively ($p < 0.05$). In contrast, DA levels in the LGD decreased from 340 ± 6 to 310 ± 6 ng/g ($p < 0.05$), while 5-HT levels in the VC decreased from 410 ± 7 to 365 ± 5 ng/g. These findings indicate differential monoaminergic responses across the central visual structures during retinal pigment dystrophy, suggesting that retinal degeneration is accompanied by region-specific alterations in central neuromodulatory mechanisms. The observed changes in 5-HT-ergic, NA-ergic, and DO-ergic systems may reflect adaptive and/or compensatory processes associated with impaired retinal input and altered

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Corresponding author: Kh. Miryusifova. Living Systems Research Institute Public Legal Entity, Ministry Science and Education Republic of Azerbaijan/ Baku State University, Baku, Azerbaijan. E-mail: miryusifovaxuraman@mail.ru

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central-peripheral visual integration. Preservation of monoaminergic homeostasis within the central visual system may therefore represent an important consideration in strategies aimed at maintaining neuronal function and visual processing under dystrophic conditions.

Keywords: retinal pigment dystrophy, visual brain structures, neuromodulatory balance disruption, experiment, retina.

Резюме

Пігментна дистрофія сітківки характеризується не лише дегенерацією периферичних фоторецепторів, але й порушенням нейромодуляторного балансу в центральних структурах зорового аналізатора.

Мета. Метою дослідження було визначення кількісних змін рівнів серотоніну (5-HT), норадреналіну (NA) та дофаміну (DA) у зоровій корі (ЗК), верхньому горбку (ВГ) та таламійних колінчастих дорсальних ядрах (ЛКД) при експериментально індукованій пігментній дистрофії сітківки (ПДС).

Методи. Дослідження проводилося на кроликах, де модель дистрофії була індукована ін'єкцією моноіодоцтової кислоти. Концентрацію моноамінів у структурах мозку визначали за допомогою флуориметричного методу аналізу.

Результати. За нормальних умов 5-HT мав найвищі значення (410 ± 7 нг/г у ЗК, 380 ± 10 нг/г у ВГ, 405 ± 5 нг/г у ЛКД). У ЗК спостерігалася помітне збільшення рівня

5-HT (420 ± 10 нг/г) та DA (390 ± 10 нг/г), тоді як у ЗК було зафіксовано зниження рівня 5-HT (365 ± 5 нг/г) та NA (320 ± 10 нг/г). У ЛКД рівень дофаміну значно знизився до 310 ± 6 нг/г ($p < 0,05$).

За експериментальної пігментної дистрофії сітківки спостерігалися значні та структурно-специфічні зміни рівня моноамінів у центральних зорових структурах. У ЗК рівні 5-HT, NA та DA збільшилися з 380 ± 10 до 420 ± 10 нг/г, з 325 ± 7 до 360 ± 10 нг/г та з 335 ± 6 до 390 ± 10 нг/г відповідно ($p < 0,05$). На противагу цьому, рівні DA в ЛКД знизилися з 340 ± 6 до 310 ± 6 нг/г ($p < 0,05$), тоді як рівні 5-HT у ЗК знизилися з 410 ± 7 до 365 ± 5 нг/г. Ці дані вказують на диференціальні моноамінергічні реакції в центральних зорових структурах під час пігментної дистрофії сітківки, що свідчить про те, що дегенерація сітківки супроводжується регіонарно-специфічними змінами в центральних нейромодуляторних механізмах. Зміни, що спостерігалися в 5-HT-ергічній, NA-ергічній та ДО-ергічній системах, можуть відображати адаптивні та/або компенсаторні процеси, пов'язані з порушенням вхідного сигналу сітківки та зміненою центрально-периферичною зоровою інтеграцією. Тому збереження моноамінергічного гомеостазу в центральній зоровій системі може бути важливим фактором у стратегіях, спрямованих на підтримку нейрональної функції та зорової обробки в дистрофічних умовах.

Ключові слова: пігментна дистрофія сітківки, зорові структури мозку, порушення нейромодуляторного балансу, експеримент, сітківка.

Introduction

The visual analyzer is not composed solely of a peripheral receptor apparatus; rather, it is a complex neural network system in which the reception, transmission, and interpretation of visual information occur through tight functional integration with multiple structures of the central nervous system (CNS). Visual signals transmitted along the retino-geniculo-cortical pathway are processed through highly organized synaptic and subcortical mechanisms, in which both cortical and subcortical structures are involved [1]. Within this integrative process, monoaminergic systems—5-HT, NA, and DA—play a crucial role in the adaptive modulation of visual function [2,3,4]. These neuromodulators act through both classical synaptic transmission and “volume transmission” mechanisms, exerting widespread regulatory effects on neuronal networks. They are fundamentally involved in optimizing the signal-to-noise ratio, regulating cortical excitability levels, and maintaining synaptic plasticity [5,6].

In neurodegenerative conditions such as retinal pigment dystrophy (RPD), degenerative changes occurring in photoreceptors and retinal pigment epithelial cells lead not only to peripheral visual impairment but also to significant functional reorganization at central visual pathways, including visual cortex (VC), colliculus superior (CS), and

lateral geniculate dorsales (LGD). Recent studies indicate that these changes are accompanied by the activation of structural and functional plasticity mechanisms within the visual system [7]. Modern neurobiological research shows that functional adaptation of the visual system in RPD and other retinal degenerations cannot be explained solely by peripheral deafferentation; rather, active top-down compensatory mechanisms at the CNS level play a crucial role. In this adaptive process, key monoaminergic structures such as the locus coeruleus (LC), raphe nuclei (nR), and substantia nigra (SN) are centrally involved in the reorganization of visual networks and optimization of sensory processing. Monoamines exert diffuse effects on large neuronal populations within the CNS via volume transmission, regulating not only local synaptic transmission but also network-level processes such as rhythm generation, oscillatory synchronization, and functional connectivity. These properties characterize monoaminergic systems as key regulatory components of neuroplastic and adaptive responses in the visual system [3].

The specific purpose of the study was to determine quantitative changes in monoamines in the VC, CS, and LGD under experimentally induced RPD.

Methods

Selection and Description of Participants / Experimental Model Development

The experiment was performed using 20 healthy male Chinchilla rabbits, 6 months of age, with a body weight of 3–3.2 kg. The animals were randomly divided into two groups: a control group ($n = 10$) and an experimental dystrophy group ($n = 10$). The animals were kept in standard vivarium conditions and had free access to food and water. Prior to the experimental procedures, the rabbits were allowed to adapt to the laboratory environment and were maintained under a controlled 12-h light/12-h dark cycle. All experimental procedures complied with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes and the relevant legislation of the Republic of Azerbaijan. The study was approved by the Institutional Animal Care and Use Committee (Approval No. 1/643-IIQD).

The RPD was induced by intravenous injection into the ear vein of monoiodoacetic acid (MIA) at a dose of 18 mg/kg (2.0% solution) [8]. This method was applied to simulate early-stage metabolic disturbances in photoreceptor and retinal pigment epithelial cells of the retina. For induction of anesthesia during the MIA injection procedure, ketamine (Calipsol) was administered at a dose of 50 mg/kg.

Data Collection and Measurements / Biochemical Determination of Monoamines

For quantitative determination of serotonin, norepinephrine, and dopamine in brain structures (VC, CS, LGD) under RPD conditions, the following sequential fluorometric protocol was applied:

Tissue Preparation and Extraction

According to the Noell method, a 1-month period was allowed for the development of moderate retinal dystrophy following the injection of MIA. Accordingly, euthanasia was performed 1 month after MIA injection, following the establishment of the retinal dystrophy model. The rabbits were deeply anesthetized with general anesthesia and euthanized by decapitation. Following decapitation, the brain was rapidly removed, dissected into the required anatomical structures, weighed, and stored at -20°C until biochemical analysis. For monoamine extraction, each tissue sample was homogenized in 3 mL of ice-cold acidified n-butanol. Homogenization was performed under cooled conditions, with the samples maintained on ice. The homogenates were centrifuged at 10,000 rpm under cooled conditions. From the resulting homogenate, 2.5 mL of the organic/supernatant phase was transferred to a clean tube, followed by the addition of 0.5 mL cysteine solution and 5 mL heptane. The samples were mixed and subsequently centrifuged at 1,500 rpm under cooled conditions to facilitate phase separation. The aqueous phase was collected and used for quantitative fluorometric determination of NA, 5-HT, DA.

The fluorometric determination was based on the method of Ciarlone, with modifications. Published applications of this method use acidified n-butanol extraction followed

by separation with heptane and fluorometric determination of 5-HT using o-phthalaldehyde and catecholamines using iodine oxidation. [9].

Norepinephrine and Dopamine Determination

For catecholamine determination, 1.0 mL of the aqueous phase was transferred to a clean tube. 0.2 mL of 0.1 M EDTA reagent was added, followed by 0.1 mL of 0.1 N iodine solution. After mixing, the reaction was allowed to proceed for 2 min at room temperature. Subsequently, 0.2 mL of alkaline sulfite reagent was added and allowed to react for 2 min, followed by the addition of 0.2 mL of 5 N acetic acid.

For the preparation of the alkaline sulfite reagent, 0.5 g of anhydrous sodium sulfite was dissolved in 2 mL of distilled water, followed by the addition of 18 mL of 5 N sodium hydroxide. This reagent was freshly prepared before use.

The reaction mixtures were placed in a boiling-water bath for 2 min, cooled, and fluorescence was measured using a Hitachi MPF-4 fluorometer. NA fluorescence was determined at an excitation wavelength of approximately 380 nm and an emission wavelength of approximately 480 nm. DO fluorescence was determined at approximately 320 nm excitation and 375 nm emission. The catecholamine concentrations were calculated using appropriate external standards.

Serotonin Determination

For serotonin determination, 0.2 mL of the aqueous phase was transferred to a clean tube and treated with 1.2 mL of o-phthalaldehyde (OPT) reagent (4 mg%). The OPT reagent was prepared by dissolving 4 mg of o-phthalaldehyde in 100 mL of 10 N hydrochloric acid.

The reaction mixture was incubated in a boiling-water bath for 10 min, cooled, and the fluorescence was measured using a Hitachi MPF-4 fluorometer. Serotonin fluorescence was measured at an excitation wavelength of approximately 360–370 nm and an emission wavelength of approximately 470 nm. The serotonin concentration was calculated from the corresponding calibration standards.

Reagent Preparation

The following reagent concentrations were used according to the Ciarlone fluorometric procedure: EDTA, 0.1 M; iodine, 0.1 N in absolute ethanol; acetic acid, 0.2 N and 5 N; sodium hydroxide, 5 N; alkaline sulfite, freshly prepared 25% (w/v); and o-phthalaldehyde (OPT), 4 mg% in 10 N hydrochloric acid. The monoamine standards were prepared in acidic solution and used for calibration of the fluorometric measurements.

All extraction and phase-separation procedures were performed under cooled conditions to minimize degradation of the monoamines. Samples were maintained on ice during tissue processing, and centrifugation was performed under cooled conditions.

Statistics

Statistical analysis and mathematical processing of the data were performed using Microsoft Excel 2019 (Microsoft Corporation, Redmond, WA, USA). One-way analysis

of variance (ANOVA) was used to assess differences among the experimental groups, and statistical significance was set at $p < 0.05$.

Results

In the first stage of the experimental study, the levels of 5-HT, NA and DA were determined in three central visual structures of the control rabbits: the VC, CS and LGD (Figure 1). In the VC, the concentrations of 5-HT, NA, and DA were 410 ± 7 , 375 ± 5 , and 395 ± 8 ng/g, respectively. In the CS, the corresponding concentrations were 380 ± 10 , 325 ± 7 , and 335 ± 6 ng/g, whereas in the LGD they were 405 ± 5 , 340 ± 7 , and 340 ± 6 ng/g, respectively. Comparative analysis showed that 5-HT had the highest concentration among the three monoamines in all three structures. The VC was characterized by the highest basal levels of NA and DA, whereas the CS generally showed the lowest monoamine concentrations; however, these differences were not statistically significant. The LGD demonstrated intermediate values.

Following induction of experimental retinal dystrophy, the concentrations of the three monoamines were determined using the same methodology (Figure 2). In the VC, the concentrations of 5-HT, NA, and DA were 365 ± 5 , 320 ± 10 , and 370 ± 4 ng/g, respectively. In the CS, the concentrations of 5-HT, NA, and DA were 420 ± 10 , 360 ± 10 , and 390 ± 10 ng/g, respectively. In the LGD, the corresponding concentrations were 390 ± 10 , 330 ± 8 , and 310 ± 6 ng/g, respectively. Compared with the control group, statistically significant changes were observed in 5-HT, NA, and DA levels in the CS, as well as in DA levels in the LGD ($p < 0.05$; Figure 2).

Comparative analysis between the control and dystrophy groups demonstrated a decrease in 5-HT concentration in the VC from 410 ± 7 to 365 ± 5 ng/g and an increase in 5-HT concentration in the CS from 380 ± 10 to 420 ± 10 ng/g. Similarly, DA concentration increased in the CS from 335 ± 6 to 390 ± 10 ng/g, while DA concentration in the LGD decreased to 310 ± 6 ng/g. NA concentration in the CS increased from 325 ± 7 to 360 ± 10 ng/g. These changes were statistically significant.

Compared with the control group, experimental animals with retinal pigment dystrophy showed a statistically significant increase in the levels of all monoamines (5-HT, NA, and DA) in the CS region ($p < 0.05$), whereas a statistically significant decrease in DA levels was observed in the LGD region ($p < 0.05$).

Discussion

This pattern of distribution in control animals confirms that monoamines exhibit specific functional activity in the respective brain structures involved in the processing and integration of visual information. In particular, the elevated monoamine concentrations

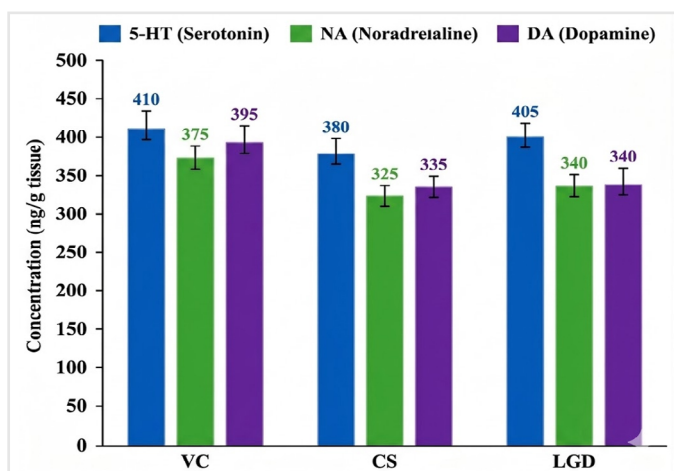


Figure 1. Basal levels of brain monoamines (serotonin (5-HT), norepinephrine (NA), and dopamine (DA)) in healthy (control) animals. (visual cortex (VC), colliculus superior (CS), lateral geniculate dorsales (LGD))

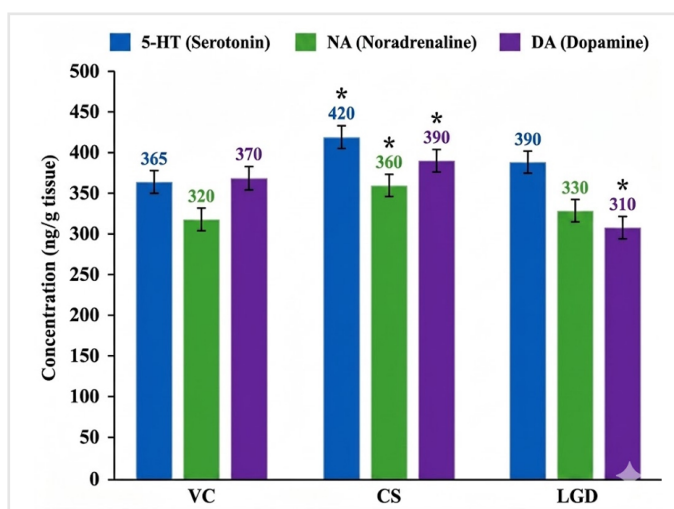


Figure 2. Concentrations of serotonin (5-HT), norepinephrine (NA), and dopamine (DA) in the visual cortex (VC), superior colliculus (CS), and lateral geniculate dorsale (LGD) following induction of experimental retinal dystrophy. Data are presented as mean $\pm$ SD. * $p < 0.05$ compared with the corresponding control group.

observed in the VC indicate a high intensity of monoaminergic modulation in the regulation of neuroplastic processes and signal transmission within this region. Under normal physiological conditions, the hierarchical distribution of monoamines observed across the VC, CS, and LGD ($VC > CS > LGD$) demonstrates structural heterogeneity of monoaminergic regulation in visual information processing.

Our results show that under dystrophic conditions, this large-scale network dynamic is significantly altered. The dynamic indicates that during dystrophy, neurotransmitter exchange between the visual centers of the brain is disrupted, and that the adaptive mechanisms of the respective structures to the pathological process differ. The observed changes in NA levels demonstrate a structure-specific pattern of noradrenergic modulation in the central visual system under experimental RPD. NA levels decreased

in the VC from 375 to 320 ng/g, whereas they increased in the CS from 325 to 360 ng/g ($p < 0.05$). This differential response may reflect distinct noradrenergic regulatory mechanisms across central visual structures in response to altered retinal input. The increase in NA in the CS may indicate enhanced noradrenergic modulation in this structure, whereas the decrease observed in the VC may reflect reduced noradrenergic signaling or altered local neurotransmitter regulation. Thus, the noradrenergic response should be considered structure-dependent rather than as a uniform increase in NA throughout the central visual system.

This represents an attempt by the noradrenergic system to regulate “synaptic gain control” in order to compensate for visual signal deficits. NA functions as an adaptive “filter” to reduce distortion of visual signals. Despite its small size, the LC has extensive projection systems, confirming its critical role in regulating cognitive and physiological arousal [10]. In our observations, the role of NA in lateral inhibition and contrast modulation indicates that this noradrenergic “reset” mechanism is essential for preserving visual information processing.

On the other hand, the relative stability or slight increase of the 5-HT system depending on the structure reflects its homeostatic modulatory and “protective” function [11]; this system provides a delayed but stable response to pathological processes. The ability of 5-HT to regulate emotional and cognitive functions within the CNS is crucial for maintaining system integrity under stress conditions associated with RPD [12,13].

One of the most significant findings of this study is the marked reduction of DA levels in the LGD (310 ng/g), while remaining relatively stable in the VC. This highlights the central role of dopamine in thalamo-cortical visual transmission and suggests that the thalamic level of visual processing is the most sensitive component in terms of dopaminergic modulation [14,15].

Several limitations of the present study should be acknowledged. First, the study was conducted using an experimental animal model of retinal pigmentary dystrophy; therefore, the findings obtained in rabbits may not be directly extrapolated to human retinal degenerative disorders. Second, the relatively limited sample size may reduce the statistical power and generalizability of the findings. Third, the study focused on quantitative changes in 5-HT, NA, and DA within selected visual brain structures, including the VC, CS, and LGD. Consequently, changes in other neurotransmitter systems and brain regions were not investigated. Finally, the fluorometric methods used for monoamine determination provide quantitative biochemical information but do not directly establish the cellular or molecular mechanisms underlying the observed changes. Further studies using larger sample sizes and complementary biochemical, histological, electrophysiological, and molecular approaches are warranted to clarify the mechanisms associated with alterations in monoamine levels following retinal pigmentary dystrophy.

To conclude, the obtained results demonstrate that under retinal pigment dystrophy, monoaminergic systems undergo differential and structure-specific reorganization at various levels of the visual analyzer. The compensatory activation of the noradrenergic system in the CS, the relative stability of the serotonergic system, and the reduction of dopaminergic modulation at the LGD level collectively indicate the formation of multi-level neuroplastic adaptation mechanisms in the visual system. These changes are associated not only with local neurotransmitter imbalance but also with large-scale network dynamics and the reorganization of neuromodulation mediated via volume transmission. Consequently, this complex interaction of monoaminergic systems can be considered one of the key compensatory mechanisms aimed at maintaining functional stability of the visual system under pathological conditions, providing a promising direction for the development of targeted neuromodulatory therapies in the future.

Author Contributions

Kh. Miryusifova: Conceptualization, methodology, writing – original draft. A. Allahverdiyeva, N. Huseynova, G. Malikova: Investigation, formal analysis. K. Cavadova, A. Huseynov, G. Abdullayeva, S. Shahmaliyeva, G. Valiyeva: Data curation, biochemical analysis, software. All authors read and approved the final version of the manuscript.

Conflict of Interest

The authors declare that they have no conflicts of interest that could influence their opinion on the subject matter or materials described and discussed in this manuscript.

Disclaimers

The opinions expressed in this article are those of the authors and do not reflect the official position of the institution.

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Ethics Statements

Subjects of the study: Animals (rabbits). Ethics approval for research involving animals: The study was conducted on animals. The experiment was performed in accordance with institutional and international standards for the care and use of laboratory animals.

Data Availability Statement

All data generated or analyzed during this study are included in this published article (within figures and text).

Abbreviations

5-HT – serotonin, NA – norepinephrine, DA – dopamine, VC – visual cortex, CS – superior colliculus, LGD – lateral geniculate dorsalis, RPD – retinal pigment dystrophy.

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