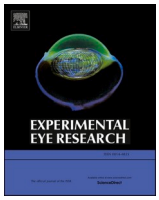
Experimental Eye Research 234 (2023) 109604

Contents lists available at ScienceDirect

Experimental Eye Research

journal homepage: www.elsevier.com/locate/yexer



Research article

Increased ocular dopamine levels in rabbits after blue light stimulation of the optic nerve head

Carlos Carpena-Torres [a], Tim Schilling [b], Fernando Huete-Toral [c], Hamed Bahmani [b], Gonzalo Carracedo [a,*]

[a] Ocupharm Research Group, Department of Optometry and Vision, Faculty of Optics and Optometry, Complutense University of Madrid, Madrid, Spain
[b] Dopavision GmbH, Berlin, Germany
[c] Ocupharm Research Group, Department of Biochemistry and Molecular Biology, Faculty of Optics and Optometry, Complutense University of Madrid, Madrid, Spain

ARTICLE INFO

Keywords:
Dopamine
Melanopsin
Intrinsically photosensitive retinal ganglion cells
Dopaminergic amacrine cells
Blue light
Myopia

ABSTRACT

The purpose was to quantify ocular dopamine in rabbits after stimulation of the optic nerve head with short-wavelength (blue) light to activate melanopsin expressed in the axons of intrinsically photosensitive retinal ganglion cells (ipRGCs). Dopamine levels in tears, aqueous humor, vitreous body, and retina (including choroid) were quantified after blue light stimulation of the optic nerve head of 15 rabbits with an optical fiber for 1 min, 10 min, or no stimulation (n = 5, each group). The left eye of all rabbits was operated on to introduce the optical fiber and stimulate the optic nerve, while the contralateral eye served as internal control. One minute of blue light stimulation significantly increased dopamine concentration in the vitreous body of the treated eyes compared to the contralateral ones ($P = 0.015$). Stimulation for 10 min significantly increased dopamine concentration in the vitreous body, as well as the aqueous humor ($P < 0.05$). Therefore, using an optical fiber approach to stimulate the optic nerve head with blue light significantly increased dopamine concentration in the aqueous humor and the vitreous body. This likely reflects an upregulation of retinal dopamine synthesis that could be attributed to ipRGC activation. However, the data provided in this study fell short of establishing a definitive link between dopamine release and ipRGC activation, mainly due to the lack of evidence supporting the expression of the melanopsin photopigment in the optic nerve.

1. Introduction

Intrinsically photosensitive retinal ganglion cells (ipRGCs) contribute to a multitude of processes and have far-reaching functional implications in the eye and brain. A specialized subset of retinal ganglion cells, ipRGCs owe many of their unique properties to the photopigment melanopsin, a light-sensitive protein encoded by the gene *Opn4* (Do, 2019). Melanopsin is most sensitive to light in the blue range of the visible spectrum ($\lambda_{peak} = \sim 480$ nm) and is expressed in the cell bodies, axons, and proximal dendrites of ipRGCs (Hattar et al., 2002). Unlike rods and cones, melanopsin allows ipRGCs to signal slow changes in ambient light conditions over prolonged periods (Do and Yau, 2010). As a result, ipRGCs have been implicated in various non-image-forming processes (Sondereker et al., 2020) – in particular, circadian entrainment and the pupil light response– since shortly after their discovery (Provencio et al., 1998).

In the eye, ipRGCs influence the pupillary light reflex, circadian regulation, and contrast sensitivity, among other functions (Sondereker et al., 2020). Beyond the eye, ipRGCs have been found to project to more than a dozen brain regions in mice, including the suprachiasmatic nucleus, olivary pretectal nucleus, and dorsal lateral geniculate nucleus (Mure, 2021). The full extent of the contributions of this small, but influential subset of retinal ganglion cells is still under study (Sondereker et al., 2020). One interaction of particular interest in the retina is that between ipRGCs and dopaminergic amacrine cells (DACs) (Zhang et al., 2008).

Located in the inner nuclear layer, DACs are the primary source of dopamine in the eye. An influential neuromodulator, dopamine influences almost all major cell types in the retina. It is well-established that dopamine plays a critical role in adapting retinal processes to changing light conditions and participates in retinal circadian regulation (Witkovsky, 2004). Dopamine has also been implicated in ocular growth

* Corresponding author. Departamento de Optometría y Visión, Facultad de Óptica y Optometría Universidad Complutense de Madrid, C/ Arcos de Jalón, 118, 28037, Madrid, Spain.
E-mail address: jgcarrac@ucm.es (G. Carracedo).

https://doi.org/10.1016/j.exer.2023.109604
Received 13 October 2022; Received in revised form 18 July 2023; Accepted 25 July 2023
Available online 26 July 2023

and development, as well as myopia progression (Martinez-Aguila et al., 2021; Zhou et al., 2017).

DACs release dopamine in response to steady (Godley and Wurtman, 1988) and flickering (Kirsch and Wagner, 1989) illumination, as well as periods of darkness (Weiler et al., 1997). There is some evidence that dopamine synthesis follows a U-shape in the mammalian retina, being stimulated under mesopic and photopic light conditions, and suppressed under scotopic conditions (Popova, 1995). All three photoreceptors –rods, cones, and ipRGCs– have been found to provide input to DACs (Roy and Field, 2019), but the precise mechanisms through which light influences dopaminergic processes in the retina remain unknown (Zhang et al., 2008).

The input ipRGCs provide to DACs is unique, traveling retrogradely from the inner to the outer retina. This signaling likely occurs via intraretinal axon collaterals (Prigge et al., 2016) that communicate ambient light information to ON-sustained DACs facilitating dopaminergic responses to gradual changes in light conditions (Zhang et al., 2008). Functionally, signaling from ipRGCs to DACs contributes to dopamine-driven light adaptation processes (Prigge et al., 2016; Sondereker et al., 2020) and retinal circadian regulation (Dkhissi-Benyahya et al., 2013). Yet, there remain questions about the interaction between ipRGCs and DACs and the influence of other photoreceptors – in particular, rods (Cameron et al., 2009; Munteanu et al., 2018; Pérez-Fernández et al., 2019) – may have on the light-mediated release of dopamine.

The present study sought to explore the impact of ipRGC stimulation on ocular dopamine in rabbits. To do so, we employed a novel fiber-optic approach to selectively stimulate the melanopsin-containing axons of ipRGCs at the optic nerve head with short-wavelength (blue) light, in order to engage the ipRGCs-DACs signaling pathway. In humans, a virtual reality approach to stimulating the optic nerve head with blue light elicited the melanopsin-driven post-illumination pupil response (PIPR) (Schilling et al., 2020) and altered retinal activity (Amorim-de-Sousa et al., 2021; Schilling et al., 2022). The current investigation directly measured the effect of blue light stimulation of the optic nerve head on dopamine in the tears, aqueous humor, vitreous body, and retina (including choroid) of rabbits. It was hypothesized that blue light stimulation of the optic nerve head using an optical fiber would significantly increase ocular dopamine relative to no light.

2. Material and methods

2.1. Study design

An experimental, short-term prospective, and randomized study was carried out in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and with the Directive 2010/63/EU of the European Parliament and the Council on the protection of animals used for scientific purposes. The study protocol was approved by the Animal Experimentation Ethics Committee of the Complutense University of Madrid (protocol code: ES280790000086).

Dopamine levels in the tears, aqueous humor, vitreous body, and retina (including choroid) of rabbits were quantified after stimulation of the optic nerve head with blue light for 1 min or 10 min, or after no light stimulation (control group). The left eye of all rabbits was the experimental eye, while the right (contralateral) eye was used as a within-group control. The experiment was performed over 15 days with 1 randomly selected rabbit being evaluated per day at the same time (10:00 a.m.) to avoid bias associated with the circadian fluctuation of dopamine.

The stimulation time was chosen based on a previous study by Amorim-de-Sousa et al. (2021) performed on humans by electroretinography, who found that blue light stimulation of the optic nerve for 1 min would be sufficient to activate the melanopsin present in the axons of ipRGCs. Thus, another group of rabbits stimulated for 10 min was added upon the possibility that stimulation for 1 min was insufficient to find measurable changes in ocular dopamine.

2.2. Animals

Fifteen male New Zealand white rabbits (n = 15) provided by the San Bernardo farm (Navarra, Spain) were used in this experiment. The rabbits were 2 months old and weighed between 3.0 and 3.5 kg at the start of the study. All animals were housed in cages under controlled 12 h light-dark cycles (light on from 8:00 a.m. to 8:00 p.m.), an ambient temperature of 18 °C, and humidity of ~40% with free access to food and water. Before the beginning of the experiment, the rabbits were accommodated in their cages for 5 days to adjust them to their new environment.

As both eyes of each rabbit were evaluated (the left eye was treated and the contralateral eye was used as an internal control), the total sample was divided into 3 groups: 5 rabbits underwent no light stimulation as a control, 5 rabbits were stimulated with blue light for 1 min, and 5 rabbits were stimulated with blue light for 10 min.

2.3. Experimental setup

Fig. 1 shows the experimental setup. The optical system (Thorlabs; Newton, New Jersey, USA) was composed of a light-emitting diode (LED) driver, a 17.2 mW blue LED with a maximum (peak) intensity at 470 nm, a 13.2 mW red LED with a maximum (peak) intensity at 631 nm (Fig. 2), an optical fiber coupled to a cannula 200 μm in diameter (0.22 NA, length = 20 mm) that reduced the LED power output to a maximum of 7 mW, and a stereotactic system. The rabbit model used was selected because the diameter of the cannula is smaller than the optic nerve head of these animals.

The control knob of the LED driver had 6 positions, corresponding to 1.2, 2.3, 3.5, 4.7, 5.8, and 7.0 mW (positions 1 to 6, respectively) through the optical fiber. The stereotactic system was composed of two translation stages with standard micrometers to direct the optical fiber in two horizontal directions, a travel vertical translation stage, and a manual rotation stage. The optical fiber was coupled to the bracket of the stereotactic system with a self-made 3D-printed piece.

The optical bench was cut to incorporate a VX75 slit lamp (Luneau Technology; Chartres, France) to observe and control the positioning of the cannula over the optic nerve head. Additionally, a lifting platform was used to place the rabbits perpendicularly to the slit lamp, inside a cage incorporating a self-made 3D-printed chinrest.

2.4. Surgical and stimulation procedures

The surgery and light stimulation procedures were performed in a dark room illuminated by a red LED (between 600 and 700 nm) to avoid influencing dopamine synthesis. The rabbits were anesthetized by an intramuscular injection of ketamine (50 mg/kg) and xylazine (5 mg/kg). Additionally, one drop of commercial topical anesthesia (Alcon Cusí; Barcelona, Spain) composed of 5 mg/ml of tetracaine chlorhydrate and 0.5 mg/ml of naphazoline chlorhydrate was instilled in each eye. After 5 min, the nictitating membrane was retracted with a surgical clamp and a hole was made in the nasal portion of the eye, at pupillary center height, with a 23G syringe. The optical fiber was then inserted into the eye using the stereotactic system. The positioning of the optical fiber over the optic nerve head was observed through the slit lamp using a 90 D non-contact slit lamp lens (Volk Optical; Mentor, Ohio, USA). The navigation of the optical fiber was guided by the red LED to avoid stimulating the retina with blue light.

Once the optical fiber was positioned over the optic nerve head, the light source was changed to the blue LED for stimulation. In the control group, the light source was switched off and the optical fiber was kept over the optic nerve head for 10 min. In the group of rabbits stimulated for 1 min, the maximum intensity of the blue LED (driver position 6) was selected. In the group of rabbits stimulated for 10 min, the intensity of

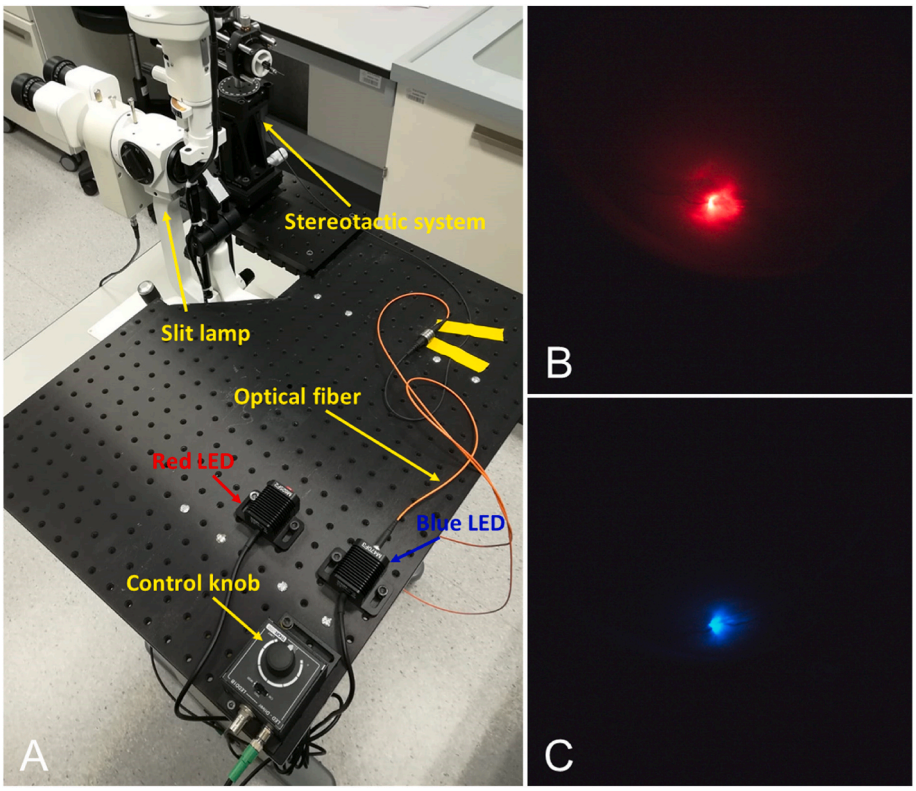


Fig. 1. Representative images of the experimental setup (a), the optical fiber during its positioning over the optic nerve head using the red LED (b), and stimulation of the optic nerve head with the blue LED (c). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

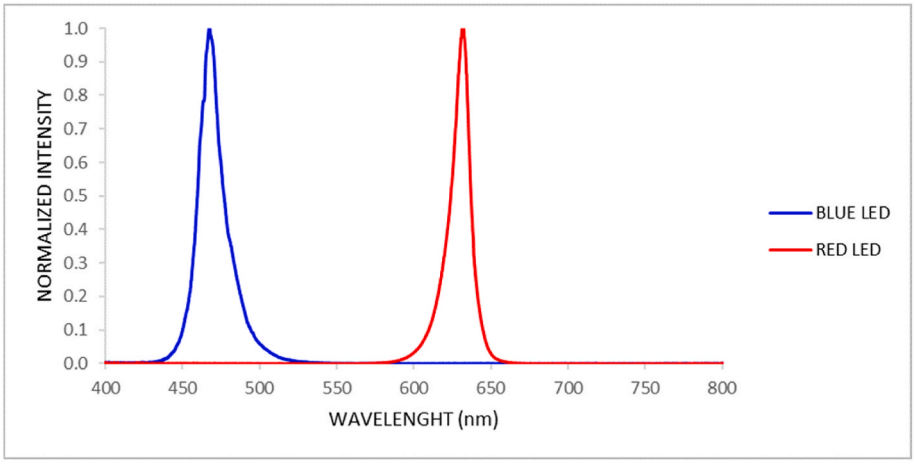


Fig. 2. Normalized light spectrum of the optical fiber-coupled LEDs that were used during the experiment. The blue LED had a maximum (peak) intensity at 470 nm, while the red LED had a maximum (peak) intensity at 631 nm. Data were provided by the manufacturer (Thorlabs; Newton, New Jersey, USA). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

the blue LED was increased every 2 min (from driver position 1 through 5).

2.5. Sample collection and processing

Twenty minutes after removing the optical fiber, the rabbits were sacrificed by an intravenous injection of sodium pentobarbital (300 mg/kg). The tears and aqueous humor were collected while the rabbits were alive, whereas the vitreous body and retina (including choroid) were collected after the rabbits were sacrificed.

The tears were collected using a Schirmer's strip (Lenticon; Madrid, Spain) for 5 min, immediately before the surgical procedure (pre-condition), as well as 20 min after light stimulation (post-condition). The Schirmer's strips were stored in Eppendorf tubes containing 200 μl of ultrapure water at −80 °C until processed. The aqueous humor was extracted with a 25G syringe to obtain 100–150 μl from each eye and was stored at −80 °C.

When the rabbits were sacrificed, their eyeballs were enucleated with a scalpel. The vitreous body was extracted with a 20G syringe to obtain 200–250 μl from each eye. The retina and choroid were separated from the sclera using a scalpel and were stored at −80 °C in a single Eppendorf tube containing 500 μl of ultrapure water until processed.

The retina was not separated from the choroid. Subsequent references to the retina refer to both the retina and the choroid.

For processing, all samples stored in Eppendorf tubes were vortexed for 5 min. In the case of the tears, the Schirmer's strip was removed after this step. Next, all the samples were heated in a dry bath at 98 °C for 2 min and then immediately immersed in an ice bath for 5 min. Subsequently, the samples were centrifuged at 15000 rpm and 4 °C for 10 min. Finally, the supernatant was transferred to a new vial to be analyzed in the high-performance liquid chromatography (HPLC) system. This processing method degraded 2.7% of the standard dopamine samples measured by HPLC. In addition, the retinal samples were not homogenized because this procedure generated other analytes interfering with the HPLC signal for dopamine quantification by the protocol described in the following section.

2.6. Analysis by high-performance liquid chromatography

The levels of dopamine in the tears, aqueous humor, vitreous, and retina (including choroid) were quantified using the Agilent 1260 HPLC system (Agilent Technologies; California, USA). A C18 KromaPhase column (Scharlab; Barcelona, Spain) with a particle size of 5 μm, a pore size of 100 Å, a length of 250 mm, and a diameter of 4.6 mm was used.

To quantify dopamine amount following the protocol described by Muzzi et al. (2008), the mobile phase was composed of 0.02 M potassium phosphate buffer (Merck; Darmstadt, Germany) at pH 2.5 (A) and methanol (B). The composition of the mobile phase was changed according to this gradient: from 0 to 10 min with 97% (A) and 3% (B), from 10 to 14 min with 80% (A) and 20% (B), from 14 to 22 min with 97% (A) and 3% (B), and the run finished at 30 min. The flow rate was 1 ml/min and the temperature was maintained at 22 °C. Dopamine was detected by injecting a total volume of 100 μl per sample and measuring the UV absorbance at 230 nm. This method of detection presented a sensitivity for dopamine quantification of 0.3 pmol (Fig. 3).

The dopamine amount in the retinal samples (in nmol) was normalized to the total DNA weight (in μg) present in each of these samples. This normalization was necessary because the quantity of extracted retina varied for each eye (depending on the size of each eye). Thus, the dopamine amount was normalized to the DNA weight since the DNA weight is directly associated with the quantity of extracted retina. Consequently, the values for retinal dopamine were reported as nmol of dopamine per μg of DNA (nmol/μg_{DNA}). For example, a retinal sample had a dopamine amount of 161.3 nmol and a DNA weight of 113 μg, resulting in a dopamine amount of 1.43 nmol per μg of DNA (1.43 nmol/μg_{DNA}). The DNA weight was measured using spectrophotometry with the NanoDrop 2000 system (Thermo Fisher Scientific; Waltham, Massachusetts, USA).

The units for dopamine amount in the tears and the concentration of dopamine in the aqueous humor and vitreous are nmol and μM, respectively.

2.7. Statistical analysis

Statistical analyses were performed using the SPSS Statistics 23 software (IBM; Chicago, Illinois, USA). The normality of the statistical variables was assessed using the Shapiro-Wilk test. Between-group statistical comparisons of the treated eye (left) or the contralateral eye (right) were performed using the Student's t-test for independent samples (normal distributions) or the Mann-Whitney *U* test (non-normal distributions). Additionally, within each group, statistical comparisons between the treated eye and the contralateral eye were performed using the Student's t-test for related samples (normal distributions) or the Wilcoxon signed-rank test (non-normal distributions). For the tears, the tests for related samples were also applied to compare baseline values (pre-condition) to values after stimulation (post-condition). A statistical significance level of 95% was established ($p < 0.05$).

3. Results

Dopamine levels in the tears, aqueous humor, vitreous body, and retina were analyzed in the treated and contralateral eyes of the rabbits. The treated eyes correspond to both the eyes that were operated on but not stimulated (control group), as well as those that were stimulated with blue light for 1 min or 10 min. The contralateral eyes were neither operated on nor stimulated. The results of dopamine levels in the tears (Fig. 4.a.) and retina (Fig. 4.d.) showed no statistically significant differences between the three groups or between the treated and contralateral eyes within each group ($p \geq 0.05$). Besides, there were no differences between pre-condition and post-condition in tears ($p \geq 0.05$). On the other hand, stimulation of the optic nerve head with blue light for 10 min significantly increased the dopamine concentration in the aqueous humor (Fig. 4.b.) and vitreous body (Fig. 4.c.) when the treated and contralateral eyes were compared ($p < 0.05$). A significant increase in dopamine concentration was also found in the vitreous body after stimulation for 1 min ($p = 0.015$). Finally, there were no statistical differences in dopamine in the tears, aqueous humor, vitreous body, or retina between the treated and contralateral eyes in the control group ($p \geq 0.05$).

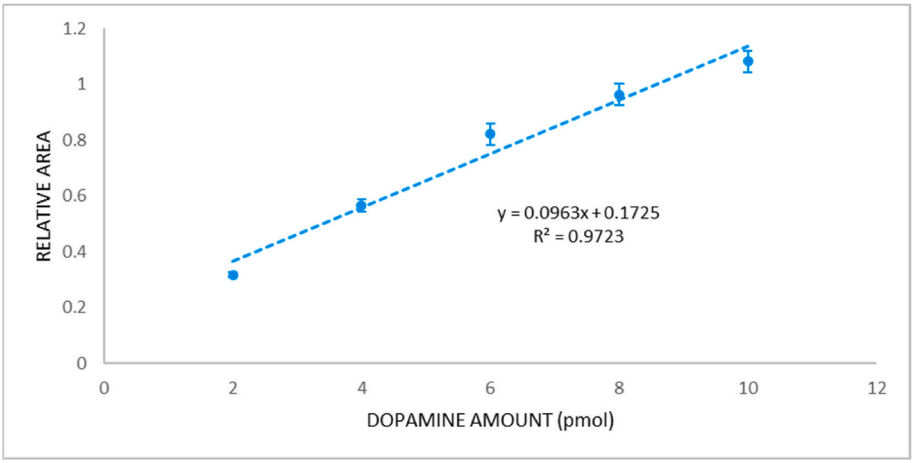


Fig. 3. Calibration curve to calculate the dopamine limit of detection by the Agilent 1260 HPLC system and the protocol described by Muzzi et al. (2008). For a minimum relative area of 0.2 μV·s, the sensitivity for dopamine quantification was established at 0.3 pmol ($n = 3$, each point).

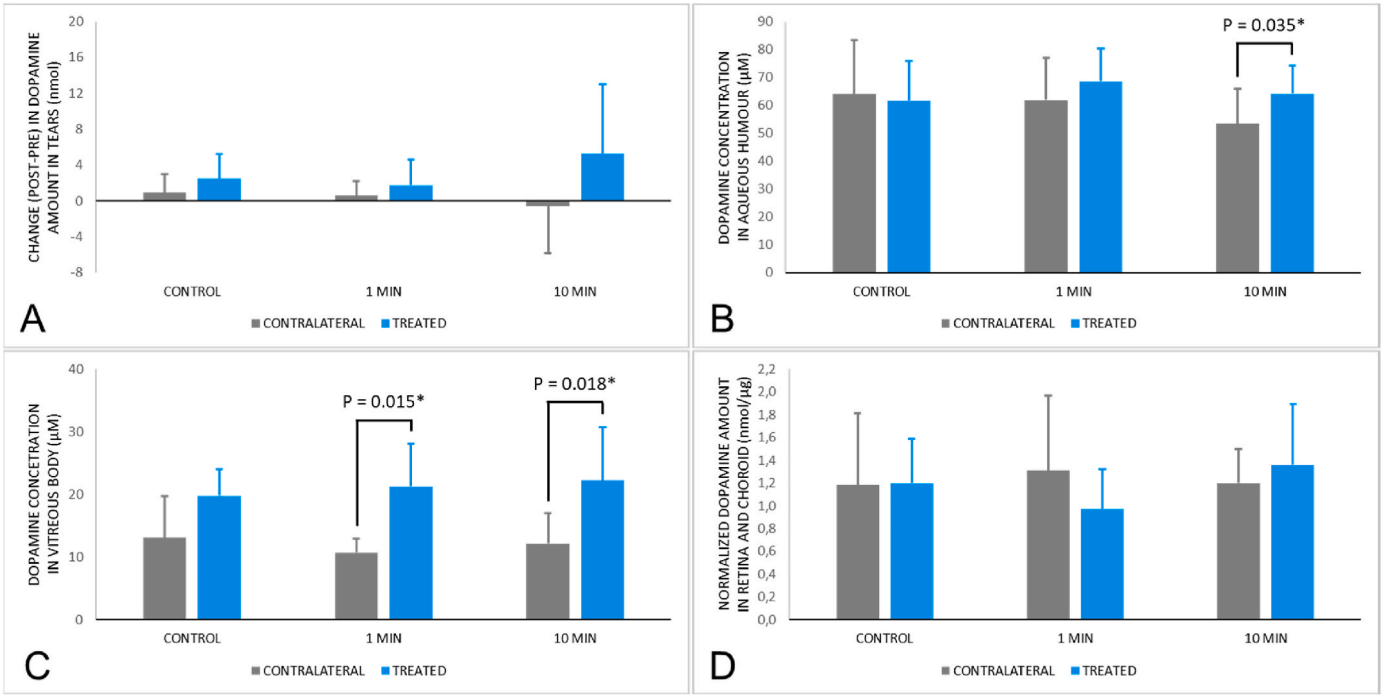


Fig. 4. Average change in dopamine (nmol) in the tears (a), average dopamine concentration (µM) in the aqueous humor (b), average dopamine concentration (µM) in the vitreous body (c), and normalized dopamine content (nmol/μg_{DNA}) in the retina (including choroid) (d) after blue light stimulation of the optic nerve head for 1 min, 10 min, or with no stimulation (n = 5, each group) are shown. $^*p < 0.05$, Student's t-test for related samples, comparison between the contralateral and treated eyes. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

4. Discussion

To our knowledge, this is the first study to investigate the effect of selective optic nerve head stimulation with short-wavelength (blue) light on ocular dopamine levels. In rabbits, the stimulation of the optic nerve head with blue light was accompanied by a significant increase in dopamine concentration in the vitreous body and aqueous humor. This stimulation did not result in measurable changes in dopamine in the tears or retina. In the control group, the surgical insertion of the optical fiber without light stimulation was not associated with significant changes in dopamine in the tears, aqueous humor, vitreous body, or retina.

In the vitreous body, selective stimulation of the optic nerve head with blue light for 1 min significantly increased the concentration of dopamine relative to no light stimulation. After 10 min of blue light stimulation, the increase in dopamine concentration was also significant in the aqueous humor. The elevated concentration of dopamine in these structures was not accompanied by a measurable change in retinal dopamine content. Retinal dopamine levels reflect the total pooled dopamine in the retina and themselves do not provide meaningful information about changes in retinal dopamine release (Feldkaemper and Schaeffel, 2013; Megaw et al., 1997). The rate of dopamine synthesis is closely tied to the demand, such that a change in dopamine synthesis may not be reflected in the tissue (retinal) dopamine levels (Megaw et al., 2001).

Rather, previous research established that vitreal dopamine concentration is a better indication of retinal dopamine release (Megaw et al., 2001; Ohngemach et al., 1997). The vitreous body is unable to synthesize or degrade dopamine or its metabolites and thus reflects the diffusion of these compounds from the retina (Megaw et al., 2001). Consistent with the light-mediated release of retinal dopamine, earlier work has demonstrated that vitreal dopamine increases in response to light exposure (Megaw et al., 1997, 2001). Indeed, the magnitude of change in vitreal content in response to light tends to be greater than that of retinal content (Parkinson and Rando, 1983). As a result, the elevated dopamine concentrations measured in the vitreous body and aqueous humor following blue light stimulation of the optic nerve head may be attributed to an increase in retinal dopamine release. In sum, as the retina is, to our knowledge, the only source of dopamine in the eye, the absence of a significant change in the total amount of retinal dopamine at the time of sampling is speculated to reflect a combination of diffusion and metabolism of the dopamine released in the retina in response to the blue light stimulation. Nevertheless, given the novelty of the fiber-optic approach employed to selectively stimulate the melanopsin-containing axons of ipRGCs at the optic nerve head, further research is warranted to better understand the implications of engaging this pathway on dopamine activity in the eye. Indeed, the light-mediated release of dopamine in the eye is an active area of research, and there are multiple hypotheses of the underlying mechanisms, including the rod activation hypothesis of light-dependent retinal dopamine release (Pérez-Fernández et al., 2019).

At this time, it cannot be ruled out the possibility that the residual activation of melanopsin, which is also present in other ocular structures such as the lens and ciliary body (Alkozi and Pintor, 2015; Alkozi et al., 2017), could mediate dopamine synthesis in the ocular anterior segment and synergistically increase dopamine release to the aqueous humor and vitreous body. However, despite the role of melanopsin in mediating melatonin production in the anterior segment having been studied over the past decade (Alkozi and Pintor, 2015; Alkozi et al., 2017), the mechanisms by which stimulation of melanopsin could modulate dopamine synthesis in the anterior segment (as suggested by the increase in dopamine concentration in the aqueous humor) or whether melanopsin is truly responsible for such modulation are still unknown.

We attribute this upregulation of dopamine synthesis to ipRGC input to DACs for several reasons. First, the wavelength of the blue LED (λ_{peak} = 470 nm) used in the treatment groups approximated the peak absorption of melanopsin (λ_{peak} = 480 nm) (Berson et al., 2002). This light source was carefully positioned using an optical fiber over the optic nerve head where the melanopsin-expressing axons of ipRGCs are the only light-absorbing structure. As a result, we can be reasonably certain

that ipRGCs were the predominant photoreceptors receiving light input and thus the only source of light information to DACs. This approach to engaging the ipRGC-DAC signaling pathway via the optic nerve head is supported by previous research employing a virtual reality system to selectively stimulate the optic nerve head of young adults (Amorim-de-Sousa et al., 2021; Schilling et al., 2022). The results of Amorim-de-Sousa et al. (2021) and Schilling et al. (2022) showed that blue light stimulation of the optic nerve led to an increase in the amplitude of the electroretinogram (ERG) b-wave. The ERG b-wave reflects electrical activity in the inner plexiform layer, where ipRGCs and DACs synapse. Additionally, no changes were observed in the amplitude of the ERG a-wave, which is associated with photoreceptor action potentials. Nevertheless, further research will be important to better understand the signaling mechanisms leading to light-induced dopamine release in the retina and the potentially varied influences of the rod, cone, and ipRGC input (Zhao et al., 2017). In this regard, the findings of the present study contradicted part of the hypothesis proposed by Pérez-Fernández et al. (2019) in rodents. Their study found no involvement of ipRGCs in retinal dopamine release, but our study did not rule out the possibility that rod activation also mediates this dopamine release in rabbits. It should be noted that rodents, rabbits, and humans may have different or multiple mechanisms for light-activated dopamine release, but the experimental approach of the present study did not provide sufficient evidence to draw a definitive conclusion regarding these mechanisms.

Knowledge of the interactions between ipRGCs and DACs, and the role of light in this signaling pathway, may have implications for various fields of research. Both ipRGCs (Do, 2019; Sondereker et al., 2020) and dopamine (Witkovsky, 2004) participate in several key retinal processes, including circadian regulation and retinal light-adaptation, which contribute to visual function. Melanopsin (Chakraborty et al., 2021), retinal dopamine (Zhou et al., 2017), and light (Jiang et al., 2018) have also been implicated in ocular growth and myopia development. There is evidence that the rate of dopamine release, rather than overall retinal dopamine content, may be the signal that controls ocular growth (Ohngemach et al., 1997). In this case, understanding the light-mediated signaling mechanisms that influence dopamine release in the retina, including if this involves ipRGC input, could be crucial to advancing scientific understanding of myopia development and control of its progression.

Finally, while the current study included a no light stimulation control group, mid- (green) and long-wavelength (red) light-stimulation control groups would provide a better understanding of any influence of other photoreceptors on the modulation of ocular dopamine in future studies. Additionally, while the optical fiber stimulation approach was carefully designed to minimize scattered light (in particular, by circumventing the cornea and lens, two major sources of intraocular light scatter (van den Berg, 1995) and illuminate only the optic nerve head, the inadvertent activation of optic nerve-peripheral melanopsin due to light diffusion cannot be completely ruled out. Light reflection in the eye may have been also exacerbated by the use of albino rabbits, which do not have a retinal pigment epithelium. On the other hand, direct comparison with other studies in the scientific literature has some limitations due to methodological differences in the species used, sample collection and processing, or data analysis, among other aspects.

5. Conclusions

Selective stimulation of the optic nerve head with short-wavelength (blue) light was associated with significantly elevated dopamine concentrations in the aqueous humor and vitreous body compared to no light stimulation. The increase in dopamine concentration observed in these structures could be attributed to an upregulation of dopamine release in the retina. The novel stimulation approach used here allowed us to hypothesize that ipRGC input to DACs would be responsible for the increase in retinal dopamine synthesis. This study contributes to the ongoing effort to understand the mechanisms through which light mediates retinal dopamine activity and the involvement of ipRGCs in these processes. However, the data provided in this study fell short of establishing a definitive link between dopamine release and ipRGC activation, mainly due to the lack of evidence supporting the expression of the melanopsin photopigment in the optic nerve. More research is necessary to elucidate the physiological mechanisms by which melanopsin is stimulated, how ipRGCs and DACs communicate, the impact this interaction has on the retinal dopaminergic system, and the broader implications of ipRGC-mediated light-driven dopamine production on retinal function and ocular growth regulation for controlling myopia progression.

Funding

This work was supported by the Federal Ministry of Education and Research, Industrie-in-Klinik-Plattform Program BMBF, Germany (FKZ: 13GW0256).

Declaration of competing interest

Hamed Bahmani and Tim Schilling are employees of Dopavision GmbH. Hamed Bahmani is also named as co-inventor for a patent application owned by Dopavision GmbH (WO2018/224671) which relates to a system and method to stimulate the optic nerve head. The remaining authors have no financial or intellectual property interest in any material or method mentioned.

Data availability

Data will be made available on request.

Acknowledgments

The authors want to thank Miguel Ángel Antón Revilla for his technical support in developing the stereotactic system to stimulate the optic nerve head and Nikita Wong for her assistance in preparing the manuscript.

References

Alkozi, H.A., Pintor, J., 2015. TRPV4 activation triggers the release of melatonin from human non-pigmented ciliary epithelial cells. Exp. Eye Res. 136, 34–37.
Alkozi, H.A., Wang, X., Perez de Lara, M.J., Pintor, J., 2017. Presence of melanopsin in human crystalline lens epithelial cells and its role in melatonin synthesis. Exp. Eye Res. 154, 168–176.
Amorim-de-Sousa, A., Schilling, T., Fernandes, P., Seshadri, Y., Bahmani, H., González-Méijome, J.M., 2021. Blue light blind-spot stimulation upregulates b-wave and pattern ERG activity in myopes. Sci. Rep. 11, 9273.
Berson, D.M., Dunn, F.A., Takao, M., 2002. Phototransduction by retinal ganglion cells that set the circadian clock. Science 295, 1070–1073.
Cameron, M.A., Pozdeyev, N., Vugler, A.A., Cooper, H., Iuvone, P.M., Lucas, R.J., 2009. Light regulation of retinal dopamine that is independent of melanopsin phototransduction. Eur. J. Neurosci. 29, 761–767.
Chakraborty, R., Landis, E.G., Mazade, R., Yang, V., Strickland, R., Hattar, S., Stone, R.A., Iuvone, P.M., Pardue, M.T., 2021. Melanopsin modulates refractive development and myopia. Exp. Eye Res. 214, 108866.
Dkhissi-Benyahya, O., Coutanson, C., Knoblauch, K., Lahouaoui, H., Leviel, V., Rey, C., Bennis, M., Cooper, H.M., 2013. The absence of melanopsin alters retinal clock function and dopamine regulation by light. Cell. Mol. Life Sci. 70, 3435–3447.
Do, M.T., Yau, K.W., 2010. Intrinsically photosensitive retinal ganglion cells. Physiol. Rev. 90, 1547–1581.
Do, M.T.H., 2019. Melanopsin and the intrinsically photosensitive retinal ganglion cells: biophysics to behavior. Neuron 104, 205–226.
Feldkaemper, M., Schaeffel, F., 2013. An updated view on the role of dopamine in myopia. Exp. Eye Res. 114, 106–119.
Godley, B.F., Wurtman, R.J., 1988. Release of endogenous dopamine from the superfused rabbit retina in vitro: effect of light stimulation. Brain Res. 452, 393–395.
Hattar, S., Liao, H.W., Takao, M., Berson, D.M., Yau, K.W., 2002. Melanopsin-containing retinal ganglion cells: architecture, projections, and intrinsic photosensitivity. Science 295, 1065–1070.
Jiang, X., Kurihara, T., Torii, H., Tsubota, K., 2018. Progress and control of myopia by light environments. Eye Contact Lens 44, 273–278.

Kirsch, M., Wagner, H.J., 1989. Release pattern of endogenous dopamine in teleost retinae during light adaptation and pharmacological stimulation. Vision Res 29, 147–154.
Martinez-Aguila, A., Martin-Gil, A., Carpena-Torres, C., Pastrana, C., Carracedo, G., 2021. Influence of circadian rhythm in the eye: significance of melatonin in glaucoma. Biomolecules 11.
Megaw, P., Morgan, I., Boelen, M., 2001. Vitreal dihydroxyphenylacetic acid (DOPAC) as an index of retinal dopamine release. J. Neurochem. 76, 1636–1644.
Megaw, P.L., Morgan, I.G., Boelen, M.K., 1997. Dopaminergic behaviour in chicken retina and the effect of form deprivation. Aust. N. Z. J. Ophthalmol. 25 (Suppl. 1), S76–S78.
Munteanu, T., Noronha, K.J., Leung, A.C., Pan, S., Lucas, J.A., Schmidt, T.M., 2018. Light-dependent pathways for dopaminergic amacrine cell development and function. Elife 7.
Mure, L.S., 2021. Intrinsically photosensitive retinal ganglion cells of the human retina. Front. Neurol. 12, 636330.
Muzzi, C., Bertocci, E., Terzuoli, L., Porcelli, B., Ciari, I., Pagani, R., Guerranti, R., 2008. Simultaneous determination of serum concentrations of levodopa, dopamine, 3-O-methyldopa and alpha-methyldopa by HPLC. Biomed. Pharmacother. 62, 253–258.
Ohngemach, S., Hagel, G., Schaeffel, F., 1997. Concentrations of biogenic amines in fundal layers in chickens with normal visual experience, deprivation, and after reserpine application. Vis. Neurosci. 14, 493–505.
Parkinson, D., Rando, R.R., 1983. Effects of light on dopamine metabolism in the chick retina. J. Neurochem. 40, 39–46.
Pérez-Fernández, V., Milosavljevic, N., Allen, A.E., Vessey, K.A., Jobling, A.I., Fletcher, E. L., Breen, P.P., Morley, J.W., Cameron, M.A., 2019. Rod photoreceptor activation alone defines the release of dopamine in the retina. Curr. Biol. 29, 763–774.e765.
Popova, E., 1995. Role of dopamine in retinal function. In: Kolb, H., Fernandez, E., Nelson, R. (Eds.), Webvision: the Organization of the Retina and Visual System. University of Utah Health Sciences Center, Salt Lake City (UT). Copyright: © 2022 Webvision.
Prigge, C.L., Yeh, P.T., Liou, N.F., Lee, C.C., You, S.F., Liu, L.L., McNeill, D.S., Chew, K.S., Hattar, S., Chen, S.K., Zhang, D.Q., 2016. M1 ipRGCs influence visual function through retrograde signaling in the retina. J. Neurosci. 36, 7184–7197.
Provencio, I., Jiang, G., De Grip, W.J., Hayes, W.P., Rollag, M.D., 1998. Melanopsin: an opsin in melanophores, brain, and eye. Proc. Natl. Acad. Sci. U. S. A. 95, 340–345.
Roy, S., Field, G.D., 2019. Dopaminergic modulation of retinal processing from starlight to sunlight. J. Pharmacol. Sci. 140, 86–93.
Schilling, T., Amorim-de-Sousa, A., Wong, N.A., Bahmani, H., González-Méijome, J., Fernandes, P., 2022. Increase in b-wave amplitude after light stimulation of the blind spot is positively correlated with the axial length of myopic individuals. Sci. Rep. 12, 4785.
Schilling, T., Soltanlou, M., Seshadri, Y., Nuerk, H.C., Bahmani, H., 2020. Blue light and melanopsin contribution to the pupil constriction in the blind-spot, parafovea and periphery. In: Proceedings of the 13th International Joint Conference on Biomedical Engineering Systems and Technologies, vol. 5. Healthinf, pp. 482–489.
Sondereker, K.B., Stabio, M.E., Renna, J.M., 2020. Crosstalk: the diversity of melanopsin ganglion cell types has begun to challenge the canonical divide between image-forming and non-image-forming vision. J. Comp. Neurol. 528, 2044–2067.
van den Berg, T.J., 1995. Analysis of intraocular straylight, especially in relation to age. Optom. Vis. Sci. 72, 52–59.
Weiler, R., Baldridge, W.H., Mangel, S.C., Dowling, J.E., 1997. Modulation of endogenous dopamine release in the fish retina by light and prolonged darkness. Vis. Neurosci. 14, 351–356.
Witkovsky, P., 2004. Dopamine and retinal function. Doc. Ophthalmol. 108, 17–40.
Zhang, D.Q., Wong, K.Y., Sollars, P.J., Berson, D.M., Pickard, G.E., McMahon, D.G., 2008. Intraretinal signaling by ganglion cell photoreceptors to dopaminergic amacrine neurons. Proc. Natl. Acad. Sci. U. S. A. 105, 14181–14186.
Zhao, X., Wong, K.Y., Zhang, D.Q., 2017. Mapping physiological inputs from multiple photoreceptor systems to dopaminergic amacrine cells in the mouse retina. Sci. Rep. 7, 7920.
Zhou, X., Pardue, M.T., Iuvone, P.M., Qu, J., 2017. Dopamine signaling and myopia development: what are the key challenges. Prog. Retin. Eye Res. 61, 60–71.