The effect of pupil size on stimulation of the melanopsin containing retinal ganglion cells, as evaluated by monochromatic pupillometry

Claus Nissen*, Birgit Sander and Henrik Lund-Andersen

Department of Ophthalmology, Glostrup Hospital, University of Copenhagen, Copenhagen, Denmark

INTRODUCTION

The pupillary light reflex (henceforth termed pupillary response) is a physiological reaction of the eye, which is elicited routinely in the physical examination of patients as a marker of the function of the retina, the optic nerve, and the brain stem (Kawasaki, 1999).

Recently it was shown that a subset of retinal ganglion cells in mammals, not comprising more than approximately 1/2% of the total ganglion cell number, governs the pupillary response and entrains the daily rhythm of the organism according to the prevailing luminance and wavelength of ambient light (Berson, 2003). These cells which are intrinsically photosensitive to blue light has been termed ipRGCs (Hattar et al., 2002).

Methods and equipment based upon pupillometry have been developed to distinguish between the function of these cells and that of the rod and cone system in pupillometry (Gamlin et al., 2007), some on the undilated (Kardon et al., 2009). The consensus pupillary diameter in the right eye was continuously measured before, during, and after light exposure. Subsequently, Tropicamide 1% or Pilocarpine 2% was instilled into the left eye and when the pupil was either maximally dilated or contracted, the entire sequence of red and blue light exposure repeated. After at least 3 days, when the effect of the eye drop had subsided, the entire experiment was repeated, this time employing the other substance.

RESULTS: Prior dilatation of the left pupil augmented the post light contraction to blue (p < 0.0001), but not to red light. The contraction during light exposure did not change. Prior contraction of the left pupil decreased the post-stimulus contraction to blue light (p < 0.04).

CONCLUSION: The size of the light exposed pupil influences the magnitude of the response to blue, but not to red light. Prior dilatation may therefore prove useful, when the response to blue light – as a marker of melanopsin containing retinal ganglion cell function – is of interest, especially when this response is weak.

Keywords: the pupillary light reflex, dilatation, melanopsin, retinal ganglion cells

MATERIALS AND METHODS

SUBJECTS

Ten healthy individuals, seven women and three men (mean age of 34 years, range 22–52 years) participated in the study. Prior eye-examination, including pupil function, slit lamp examination, fundus examination, applanation tonometry, OCT (Cirrus, Humphrey Instruments, CA, USA), and autokerimetry (Humphrey Instruments, Type 750, CA, USA) revealed neither eye disease nor shallow chambers (precipitating angle closure glaucoma in pupil dilatation). None received any medication known to influence the eyes, the central, or the peripheral nervous system. All subjects participating were informed of the procedure and their written consent obtained. The rules of the Helsinki Declaration were adhered to and the study approved by the local ethics committee.

PUPILLOMETER

The pupillometer of Herbst et al. (2011) has previously been described together with the procedure used.

The instrument consists of two parts: an input section, which stimulates one eye for a predetermined time period (usually 20 s) with light of a well defined wavelength and luminance, and an
output section, detecting the area of the contralateral pupil before, during, and after light stimulation. Both sections are controlled by a common computer program and thus synchronized. The area of the pupil is monitored with a frequency of 20 Hz and converted into a diameter, assuming a circular pupil. Light intensity (luminance) was 300 cd/m² for red and blue light, corresponding to $2.2 \times 10^{15}$ quanta/cm²/s (red) and $1.7 \times 10^{15}$ quanta/cm²/s (blue) and less for the infrared detecting system. All intensities were chosen below the recommendations of ANSI-2007 and ICNIRP.

**EXAMINATION PROCEDURE**

Sessions were performed in a dark room, in which luminance was controlled by the investigator. All sessions were performed between 9 am and 4 pm in the months October to March. The left eye was exposed to light as described below and the pupil of the right eye video filmed. While the subject was seated, the instrument adjusted, ambient light was mesopic for approximately 5 min. Then, prior to examination the subject was exposed to darkness for 1 min. The examination session was as follows: 10 s of darkness (baseline pupil), 20 s of exposure to (red or blue) light, and 60 s of darkness (post-exposure). After 5–7 min the entire session was repeated. First examination session was always performed with red light 300 cd/m²; second always with blue light 300 cd/m². Subsequently either pilocarpine 2% or tropicamide 1% was instilled into the left eye and, after 20 min, the red and blue sessions repeated. After 3–14 days the entire experiment was repeated, this time comprising instillation of pilocarpine, if the first sequence had comprised tropicamide, and vice versa.

The absolute diameter of the input pupil in the contracted or dilated state was measured in the slit lamp.

**PROCESSING AND CALCULATION OF THE OUTPUT DATA**

The diameter of the right pupil was the principal output parameter. A baseline pupil diameter was calculated as the mean of determinations in the 10 s in the dark preceding light initiation. The pupillary response was expressed relative to the baseline pupil and the resultant pupillogram analyzed during the light on phase and the light off phase with the following parameters:

1. Maximal contraction amplitude (CA) defined as the maximal contraction of the pupil within the first 6 s of light exposure as % of the baseline diameter.
2. The time period (expressed in s) from light on to maximal CA (time to max).
3. Sustained CA was calculated as the area under the curve (AUC; see below) of the last second of the light on phase (the 20th second).
4. The AUC, which may be taken as the response vs. time of all contraction during a well defined time period, in this case three different periods: (1) during the 20-s of light on ($AUC_{0-20s}$ light on), (2) for the first 10 s after light is turned off ($AUC_{0-10s}$ light off), and (3) from 10 to 30 s after light is turned off ($AUC_{10-30s}$ light off).

**STATISTICAL PROCEDURE**

Since the data could be assumed to follow a normal distribution, they were analyzed with paired $t$-tests for the difference in pupillary response between the natural state and either mydriasis (dilatation with tropicamide) or miosis (contraction due to pilocarpine). Additional comparisons between responses to red and blue light and baseline measurements were analyzed in a similar manner, $p < 0.05$ being considered statistically significant. All parameters including baseline pupil diameter were subjected to analysis for correlation with age (Pearson). Calculations were performed using SAS statistical software (SAS version 9.1., SAS Institute Inc., Cary, NC, USA).

**RESULTS**

**COMPARISON BETWEEN THE EFFECT OF RED AND BLUE LIGHT ON AUC, NO EYE DROPS INSTILLED**

During light exposure the difference between the pupillary response to red and to blue light was significant, the reaction to blue always being the bigger (Figures 1 and 2). $AUC_{0–20s}$ light on, blue light was 11–13% bigger than $AUC_{0–20s}$ light on, red light. Maximal CA and sustained CA showed an identical pattern.

After light cut off, blue $AUC_{0–10s}$ light off was 33% and $AUC_{0–30s}$ light off 37% of $AUC_{0–20s}$ light on blue. In comparison, red $AUC_{0–10s}$ light off was only 21% and $AUC_{0–30s}$ light off 13% of $AUC_{0–20s}$ light on red.

**FIGURE 1 |** Pupillary contraction to a red light stimulus (660 nm) as a function of time (s). A constant and continuous stimulus of 300 cd/m² was applied at time 0 and discontinued at the end of the 20th second. The stimulus was applied to the left eye and the consensual, right pupillary contraction recorded. The yellow graph represents contraction of the non-stimulated pupil, when the input pupil was contracted (miotic) in advance with pilocarpine, the red, when it was dilated (mydriatic) in advance with tropicamide. The average area of the input pupil to pilocarpine was 2.5 mm², and to tropicamide 44.9 mm². Although this difference represents a factor of 18 in retinal illuminance, the two graphs are virtually indistinguishable. Their reaction is independent of photon load. Both graphs exhibit rapid contraction to light and show pupillary escape. When the light stimulus is terminated, fairly rapid dilatation ensues. Maximal contraction is slightly larger in the red graph than in the yellow graph (cf. Table 1). Graphs represent mean values from 10 subjects. The pupillary contraction with the input pupil in natural state was indistinguishable from these two graphs and therefore not shown. Dashed vertical lines represent light on (0 s) and light off (20 s).
The (non-normalized) baseline pupil showed correlation with age, no other parameters did. Since the present protocol does not encompass patients was relatively small (n = 10), the data were reproducible, with unchanged baseline values for the examination before installation of eye drops and the differences in baseline comparable to same-day examinations as earlier reported (Herbst et al., 2011).

The (non-normalized) baseline pupil showed correlation with age, no other parameters did. Since the present protocol does not encompass
Table 1 | Response of the right pupil to exposure of the left pupil to either red or blue light.

| Colour (incident light) | State (left eye) | Contraction amplitude (% of baseline pupil) | Time to max | AUC (area under the curve: (1 – normalized pup. diameter) x time) |
|-------------------------|------------------|---------------------------------------------|-------------|---------------------------------------------------|
|                         |                  | Maximal | Sustained | Time (s) | 0–20s light on | 0–10s light off | 10–30s light off |
| Mean Red Natural        |                  | 54.64   | 45.68     | 3.54     | 9.42         | 1.88            | 1.34            |
| SD                      |                  | 3.73    | 5.80      | 0.64     | 0.71         | 0.46            | 0.89            |
| Mean Red Dilated        |                  | 55.30   | 46.78     | 3.52     | 9.57         | 1.99            | 1.50            |
| SD                      |                  | 4.64    | 5.71      | 0.88     | 0.87         | 0.44            | 1.07            |
| Red: p-value natural vs. dilated | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |
| Mean Blue Natural       |                  | 61.30   | 54.73     | 3.42     | 10.80        | 3.40            | 3.59            |
| SD                      |                  | 3.43    | 8.50      | 0.66     | 1.22         | 0.71            | 1.58            |
| Mean Blue Dilated       |                  | 61.54   | 59.46     | 3.62     | 11.20        | 4.37            | 7.76            |
| SD                      |                  | 3.86    | 5.96      | 0.65     | 1.09         | 0.54            | 1.44            |
| Blue: p-value natural vs. dilated | n.s. | 0.007   | n.s. | n.s. | 0.0001 | <0.0001 |
| Mean Red Natural        |                  | 56.73   | 46.09     | 3.35     | 9.79         | 2.08            | 1.07            |
| SD                      |                  | 5.23    | 7.00      | 0.88     | 0.67         | 0.36            | 0.55            |
| Mean Red Contracted     |                  | 52.24   | 44.13     | 3.41     | 9.07         | 1.90            | 1.31            |
| SD                      |                  | 75.1    | 9.86      | 0.71     | 1.51         | 0.42            | 0.72            |
| Red: p-value natural vs. contracted | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |
| Mean Blue Natural       |                  | 60.17   | 57.42     | 3.62     | 10.96        | 3.59            | 4.33            |
| SD                      |                  | 3.71    | 4.65      | 0.83     | 0.79         | 0.53            | 1.76            |
| Mean Blue Contracted    |                  | 60.42   | 54.75     | 4.14     | 10.80        | 3.26            | 3.04            |
| SD                      |                  | 3.76    | 6.59      | 0.61     | 0.91         | 0.78            | 1.92            |
| Blue: p-value natural vs. contracted | n.s. | 0.032   | n.s. | n.s. | 0.038 | 0.01 |

The left pupil was either in the natural state, dilated with tropicamide or contracted with pilocarpine. Mean and SD is given for contraction amplitudes (CA), time to maximal contraction (time to max), and the area under the curve (AUC) during and after light exposure of the right pupil.

include dark adaptation, the responses to red light probably is mainly related to the L and L–M cones, which are known to adapt very fast and bleach under persistent light. Therefore red light post illumination response would be expected to be independent of pupil size, as is indeed it was in this study. The rapid return to the dark pupil diameter illustrates that melanopsin was not stimulated to any noticeable degree by red light (Figure 1). The response to blue light is known to be due to stimulation of the photopigment melanopsin present in intrinsically light sensitive retinal ganglion cells (ipRGCs) and probably to stimulation of S-cones and rods. The hallmark of the melanopsin response is low sensitivity and a slow, but sustained response, which is present in cones and rods respectively. The hallmark of the melanopsin response is low sensitivity and a slow, but sustained response, which is present in cones and rods respectively. The response to blue light is known to be due to stimulation of the photopigment melanopsin present in intrinsically light sensitive retinal ganglion cells (ipRGCs) and probably to stimulation of S-cones and rods. The hallmark of the melanopsin response is low sensitivity and a slow, but sustained response, which is present in cones and rods. The hallmark of the melanopsin response is low sensitivity and a slow, but sustained response, which is present in cones and rods.

The retinal photon flux of blue light was 7.5 × 10^{13} quanta/s in the contracted state and 1.35 × 10^{15} quanta/s in the dilated state. It can therefore be assumed that the ipRGC-system is saturated in the dilated state and most likely also in the contracted state. Assuming linearity between input and output and cf. Figure 2 it may also be assumed that the system is saturated, even when the pupil is in the natural state.

Employing radiometric units and putting lens transmission = 1.0, the retinal photon flux of blue light was 7.5 × 10^{13} quanta/s in the contracted state and 1.35 × 10^{15} quanta/s in the dilated state. It can therefore be assumed that the ipRGC-system is saturated in the dilated state and most likely also in the contracted state. Assuming linearity between input and output and cf. Figure 2 it may also be assumed that the system is saturated, even when the pupil is in the natural state.

It may be argued, that the reaction to blue light could be caused by a direct influence of tropicamide or pilocarpine on the retina. Nothing is known on the pharmacological effect of tropicamide on the retina in vivo (Shell, 1982), but it is likely to be insignificant, since diffusion through the vitreous is very slow (Lund-Andersen and Sander, 2011) and since there is no direct vascular pathway from the anterior segment to the retina. Furthermore the blood–retina-barrier should hamper contact. As for the influence of pilocarpine only very few reports exist (e.g., Kovacik et al., 1976; Shell, 1982) which does not corroborate any effect of pilocarpine on the bovine retina. Finally, any effect of tropicamide or pilocarpine on the ipRGCs would have to be highly selective, if the different responses to red and blue should be ascribed to a direct action of these substances upon the retina.
In conclusion, the present study demonstrates that for blue light exposure, the pupillary response is correlated to the size of the stimulated pupil. Therefore dilation should be considered for protocols exploring the response of blue-sensitive, intrinsically light sensitive retinal ganglion cells, especially if maximal stimulation of this cell system is intended.

REFERENCES

Berson, D. M. (2003). Strange vision: ganglion cells as circadian photoreceptors. Trends Neurosci. 26, 314–320.

Gamlin, P. D., McDougal, D. H., Pokorny, J., Smith, V. C., Yau, K. W., and Dacey, D. M. (2007). Human and macaque pupil responses driven by melanopsin-containing retinal ganglion cells. Vision Res. 47, 946–954.

Girkin, C. A. (2003). Evaluation of the pupillary light response as an objective measure of visual function. Ophthalmol. Clin. North Am. 16, 143–153.

Güler, A. D., Ecker, J. L., Lall, G. S., Haq, S., Altimus, C. M., Liao, H. W., Barnard, A. R., Cahill, H., Badea, T. C., Zhao, H., Hankins, M. W., Berson, D. M., Lucas, R. J., Yau, K. W., and Hattar, S. (2008). Melanopsin cells are the principal conduits for rod-cone input to non-image-forming vision. Nature 453, 102–105.

Hattar, S., Liao, H. W., Takao, M., Berson, D. M., and Yau, K. W. (2002). Melanopsin-containing retinal ganglion cells: architecture, projections, and intrinsic photosensitivity. Science 295, 1065–1070.

Herbst, K., Sander, B., Milea, D., Lund-Andersen, H., and Kawasaki, A. (2011). Test-retest repeatability of the pupil light response to blue and red light stimuli in normal human eyes using a novel pupillometer. Front. Neurol. 2:10. doi:10.3389/fneur.2011.00010

Kardon, R., Anderson, S. C., Damarjian, T. G., Grace, E. M., Stone, E., and Kawasaki, A. (2009). Chromatic pupil responses: preferential activation of the melanopsin-mediated versus outer photoreceptor-mediated pupil light reflex. Ophthalmology 116, 1564–1573.

Kardon, R., Anderson, S. C., Damarjian, T. G., Grace, E. M., Stone, E., and Kawasaki, A. (2011). The influence of intrinsically-photosensitive retinal ganglion cells on the spectral sensitivity and response dynamics of the human pupillary light reflex. Vision Res. 50, 72–87.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 01 October 2011; accepted: 30 December 2011; published online: 02 February 2012.

Citation: Nissen C, Sander B and Lund-Andersen H (2012) The effect of pupil size on stimulation of the melanopsin containing retinal ganglion cells, as evaluated by monochromatic pupillometry. Front. Neur. 2:92. doi: 10.3389/fneur.2011.00092

This article was submitted to Frontiers in Neuro-ophthalmology, a Specialty of Frontiers in Neurology.

Copyright © 2012 Nissen, Sander and Lund-Andersen. This is an open-access article distributed under the terms of the Creative Commons Attribution Non Commercial License, which permits non-commercial use, distribution, and reproduction in other forums, provided the original authors and source are credited.