Introducing *PyPlr*: A versatile, integrated system of hardware and software for researching the human pupillary light reflex

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Abstract

We introduce *PyPlr –* a versatile, integrated system of hardware and software to support a broad spectrum of research applications concerning the human pupillary light reflex. Fundamentally, PyPlr is a custom Python library for integrating an eye tracker with a light source and for streamlining such processes as stimulus design, optimisation and delivery, communication with respect to timing, and extraction, cleaning, and analysis of the pupil data. We additionally describe how full-field, homogenous stimulation of the retina can be realised with a low-cost integrating sphere that serves as an alternative to a Maxwellian setup. As needs demand, users can integrate their own light source, but we provide full support for a high-end 10-primary light engine which offers advanced control over the temporal and spectral properties of light stimuli. In this paper we describe the hardware and software in detail and demonstrate its capabilities with two example applications: 1) pupillometer-style measurement and parametrisation of the pupil flash response, and 2) comparing the post-illumination pupil response to long and short wavelength light. We believe the system holds promise for researchers who would favour a flexible approach to studying the pupillary light reflex and the ability to employ a wide range of temporally and spectrally varying stimuli, including simple narrowband stimuli.

Keywords: Pupillometry, instrumentation, pupillary light reflex, ganzfeld, melanopsin, silent substitution

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**Introduction**

The pupillary light reflex (PLR) refers to the constriction and dilation of the pupil in response to changes in retinal illumination. The biological role of the PLR is to optimse retinal image quality by regulating the ammount and trajectory of light that strikes the retina (Hirata et al., 2003; McDougal & Gamlin, 2015), but it is also serves as a valuable tool for gaining insight into the integrity and activity of the autonomic nervous system (Girkin, 2003). Indeed, subjective visual assessments of the PLR, such as the swinging flashlight test (Levatin, 1959; Thompson, 1966), are still used routinely in clinical investigations to unmask afferent pupillary defects and to give vital clues as to a patient’s neurological state. Though useful in critical care, such techniques are less suited to research due to their limited sensitivity and specificity, and to the poor inter- and intraobserver reliability that exists even among specialists (Litvan et al., 2000; Meeker et al., 2005). The advent and commercial availability of video-based pupillographic techniques in the 1970s enabled researchers and clinical practitioners to base their investigations on repeatable and precise quantitative measurements of pupil size. Owing to this development, and to the dedication of scientists working in the field, the behavior of the pupil in response to light is well characterised in both health and disease (Loewenfeld, 1993).

The aperture of the pupil at any given time is determined by the tone of the *dilator* and *sphincter pupillae*—the two oponent smooth muscles of the iris. The iris sphincter receives parasympathetic innervation and is responsible for the constriction of the pupil that follows an increase in retinal illumination. When light strikes the retina, photons are absorbed by photoreceptors and the neural signal traverses a short reflex arc comprising the photoreceptor, bipolar and ganglion cells of the retina, the pretectal nucleus of the midbrain, and the Edinger-Westphal nucleus, which projects to the iris sphincter muscle via the ciliary ganglion (Hall & Chilcott, 2018). Following a brief flash of white light, a healthy pupil will begin to constrict after around 2-3 hundred ms and, after reaching peak constriction, will enter a redilation phase and return to baseline. Redilation of the pupil upon light cessation depends on two integrated processes: relaxtion of the sphincter muscle due to parasympathetic inhibition, and contraction of the dilator muscle following excitation in the sympathetic pathway. The measured PLR is typically described in terms of parameters relating to constriction latency, amplitude, velocity and acceleration. Normal ageing affects the dynamics of the flash response (Bitsios et al., 1996; Winston et al., 2019), but degrees of abnormality exist in a broad range of psychiatric, neurological and ophthalmic conditions (Chen et al., 2011; Girkin, 2003; Van Stavern et al., 2019), making the flash response an important tool in research and diagnostics (Hall & Chilcott, 2018; Troiani, 2020).

It was once assumed that the PLR was controlled entirely by the integration of signals from rod and cone photoreceptors, but we now know that steady state pupil size is largely under the influence of intrinsically photosensitive retinal ganglion cells (ipRGCs)—a sub-population of retinal ganglion cells which express the photopigment melanopsin in their axons and soma (Clarke et al., 2003; Provencio et al., 2000). ipRGCs are sensitive to high intensity, short wavelength (blue) light and control non-visual functions, such as circadian photoentrainment and pupil size (Spitschan, 2019), via direct projections to the suprachiasmatic nucleus of the hypothalamus and the olivary pretectal nucleus, respectively (Do, 2019). The post-illumination pupil response (PIPR) describes the sustained constriction of the pupil following exposure to short wavelength light, usually relative to long wavelength light, and is assumed to be a unique non-invasive signature of melanopsin processing in the human retina (Adhikari et al., 2015). Like the flash response to white light, the PIPR has been researched extensively for its potential as a biomarker in various ocular and neurodegenerative diseases (Chougule et al., 2019; Feigl & Zele, 2014).

Researching the PLR requires a system for illuminating the retina and measuring pupil size. For patient monitoring in critical care, hand-held pupillometers, like the PLR-3000 (NeurOptics, Irvine, CA), offer an attractive all-in-one solution as they are portable, reliable and easy to use (Meeker et al., 2005; Taylor et al., 2003). These ‘point-and-shoot’ devices are aimed at the eye to deliver a light stimulus and use infrared illumination, video recording and internal algorithms to provide an instantaneous readout of the pupil response. The downsides of automated pupillometers are that they are expensive and inflexible, usually offering very limited control over stimulus parameters (e.g., duration, wavelength, intensity) and in some cases no access to the raw data. This makes them less suited to application in research. Video-based eye trackers on the other hand measure pupil size as part of their gaze estimation pipeline, and are often favoured in research for their versatility, though they can also be expensive and complicated to use.

Here we describe a novel, versatile system of hardware and software to support a broad range of research applications concerning the pupillary light reflex. The system comprises a low-cost integrating sphere, a high-end spectrally tuneable light engine, a Pupil Core eye tracking headset, and a custom Python software for streamlining integration and analysis.

# Overview

Fundamentally, PyPlr is a custom Python software for integrating an eye tracker with a light source and for streamlining such processes as stimulus design, optimisation and delivery, communication with respect to timing, and extraction, cleaning, and analysis of the pupil data. The software was developed against Pupil Core (Pupil Labs, GmbH)–a versatile, open source and relatively inexpensive eye tracking ecosystem that affords real-time access to data, high sampling rates, and precise model-based 3d estimation of pupil size (see Kassner et al., 2014, for a detailed overview of the system). A Pupil Core headset is however the only hardware dependency and users can easily integrate their own light source and stimulus geometry. For our setup, we made a low cost integrating sphere to provide a full field, ‘Ganzfeld’ stimulus, and used a high-end 10 led channel light engine to allow for good control over the temporal and spectral properties of light stimuli. Here we describe the hardware and software in detail before demonstrating useage with two examples.

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Figure 1. Showing the physical setup (left) and software architecture (right).

**Hardware**

**Integrating sphere.** We designed a low-cost integrating sphere to serve as an alternative to an optically complicated Maxwellian setup. The sphere was constructed from two 45-cm diameter flanged acrylic half-domes (Project Plastics Ltd), each sprayed on the inside with multiple coats of Avian-B high reflectance paint (Avian Technologies LLC). A 28 cm opening in one of the domes serves as a viewing port to a full field, ‘Ganzfeld’, stimulus, and an additional 7 cm (~9°) opening opposite the viewing port was included for secondary stimuli (e.g., a fixation target) or to exclude the foveal macular pigment from stimulation. On the same half of the sphere as the viewing port, a 30 mm entry port for the light source was cut at an angle of 22.5-deg from the top, such that it could not be seen directly when looking straight ahead. The sphere was housed on a stabilized wooden fixing plate.

## Eye tracker. Pupil Core is a versatile, open source and relatively inexpensive eye tracking ecosystem that affords real-time access to data, high sampling rates, and precise model-based 3d estimation of pupil size (see Kassner et al., 2014, for a detailed overview of the system). The Pupil Core headset also has a forward-facing world camera which we leverage to timestamp the onset of light stimuli with good temporal accuracy. Given a suitable geometry and appropriately tuned parameters, this method of timestamping light stimuli supports seamless integration with any light source.

**Light source.** We use a Spectra Tune Lab light engine (STLAB: LEDMOTIVE, technologies) to deliver light stimuli. The STLAB is a spectrally tuneable light engine with 10 LED colour channels, capable of generating a broad range of spectral compositions. The intensity of each LED is specified with a value between 0-4095 (12-bit resolution), corresponding to the minimum and maximum output.

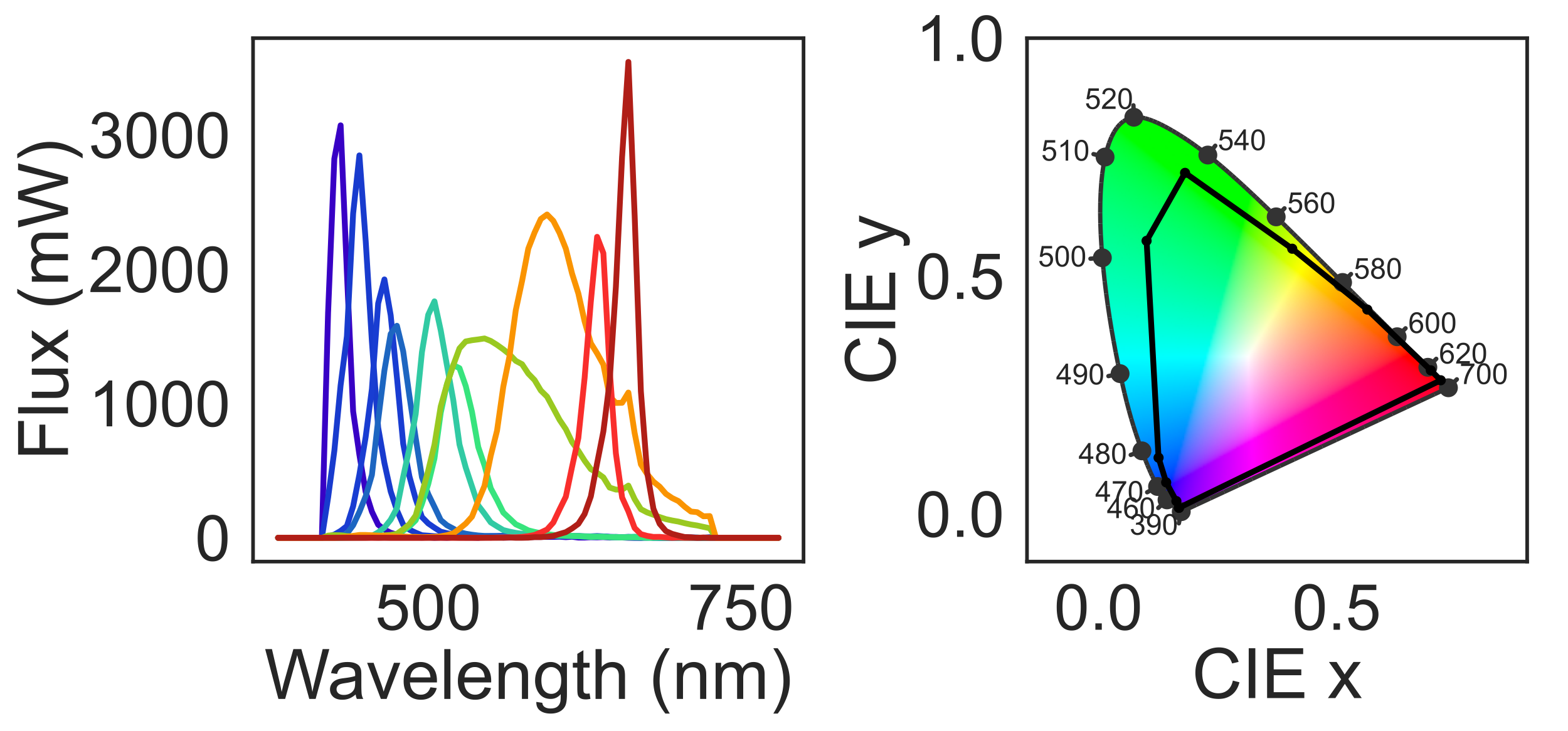


Figure 3.Spectral power distributions (left) and CIE color coordinates (right) of the 10 STLAB led channels.

**Spectrometer.** We used an OceanOptics spectrometer to obtain measurements of calibrated radiance at the viewing port. It is supported in our software, but the code can easily be modified to add support for another spectrometer.

**Software**

**Pupil Labs software**

***Pupil Capture.*** Pupil Capture is the real-time tracking application required when using the eye tracker.

***Pupil Player.*** The Pupil Labs analysis software, used for visualising, inspecting and exporting data.

***Network API.*** A scripting interface for real-time communication and data streaming. Uses the ZeroMQ messaging library and MessagePack (like JSON, but faster) for fast and reliable communication.

**PyPlr.**

## *pupil.* All of the code for communicating with the Pupil Core headset is contained within the pupil.py module. The two most important functions are *PupilCore()* and *LightStamper(). PupilCore()* simplifies connecting to the eye tracker and controlling basic functionality such as starting and stopping recording and sending event markers. *LightStamper()* issubclass of threading.Thread which has its run method overridden with code for detecting the onset of a light. Given a suitable geometry and an appropriate detection threshold, it is the basis for integrating with virtually any light source. Once initiated, the LightStamper() keeps track of the two most recent frames from the Pupil Core world camera (which must be aimed at the light source). When the average RGB difference of the two frames exceeds a given threshold, an ‘annotation’ is sent to Pupil Capture to mark the time of light onset. Figure 2 shows a minimal example of how to use the pupil module to measure the light reflex with any light source (e.g., a light switch in a dark room).

***stlab.*** Contains a class for the STLAB device which wraps its entire RESTFUL\_API. Also contains other useful functions for working with video files.

***pipeline.*** After exporting data from Pupil Player, the pipeline.py module can be used to load, clean, and extract the pupil data.

***plr.*** A module to assist with plotting and parametrisation of the PLR and PIPR.

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Figure 2. Example code showing how to use PyPlr’s pupil module to record a pupil response to any light stimulus. The result is a Pupil Labs recording with an annotation marking the time at which the light was presented.

# Examples

**Pupil flash response**

Here we show how our system can be used to measure and parametrise the pupil flash response, and we compare the results with a NeurOptics PLR-3000.

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Figure 4. Pupillometer-style measurement and parametrization of the flash response (not the final script). Produces a plot of flash response and saves parameters in csv file. This script uses the PupilGrabber() class to work with real-time pupil data and therefore bypasses the need for using the Pupil Player software.

**PIPR**

Here we describe an experiment that measures the PIPR.

**Discussion**

**References**

Adhikari, P., Zele, A. J., & Feigl, B. (2015). The post-illumination pupil response (PIPR). *Investigative Ophthalmology and Visual Science*, *56*(6), 3838–3849. https://doi.org/10.1167/iovs.14-16233

Bitsios, P., Prettyman, R., & Szabadi, E. U. (1996). Changes in autonomic function with age: A study of pupillary kinetics in healthy young and old people. *Age and Ageing*, *25*(6), 432–438. https://doi.org/10.1093/ageing/25.6.432

Chen, J., Gombart, Z., Rogers, S., Gardiner, S., Cecil, S., & Bullock, R. (2011). Pupillary reactivity as an early indicator of increased intracranial pressure: The introduction of the neurological pupil index. *Surgical Neurology International*, *2*(1), 82. https://doi.org/10.4103/2152-7806.82248

Chougule, P. S., Najjar, R. P., Finkelstein, M. T., Kandiah, N., & Milea, D. (2019). Light-induced pupillary responses in Alzheimer’s disease. *Frontiers in Neurology*, *10*(APR), 1–12. https://doi.org/10.3389/fneur.2019.00360

Clarke, R. J., Zhang, H., & Gamlin, P. D. R. (2003). Characteristics of the pupillary light reflex in the alert rhesus monkey. *Journal of Neurophysiology*, *89*(6), 3179–3189. https://doi.org/10.1152/jn.01131.2002

Do, M. T. H. (2019). Melanopsin and the intrinsically photosensitive retinal ganglion cells: biophysics to behavior. *Neuron*, *104*(2), 205–226. https://doi.org/10.1016/j.neuron.2019.07.016

Feigl, B., & Zele, A. J. (2014). Melanopsin-expressing intrinsically photosensitive retinal ganglion cells in retinal disease. *Optometry and Vision Science*, *91*(8), 894–903. https://doi.org/10.1097/OPX.0000000000000284

Girkin, C. A. (2003). Evaluation of the pupillary light response as an objective measure of visual function. *Ophthalmology Clinics of North America*, *16*(2), 143–153. https://doi.org/10.1016/S0896-1549(03)00002-6

Hall, C. A., & Chilcott, R. P. (2018). Eyeing up the future of the pupillary light reflex in neurodiagnostics. *Diagnostics*, *8*(1). https://doi.org/10.3390/diagnostics8010019

Hirata, Y., Yamaji, K., Sakai, H., & Usui, S. (2003). Function of the pupil in vision and information capacity of retinal image. *Systems and Computers in Japan*, *34*(9), 48–57. https://doi.org/10.1002/scj.10344

Kassner, M., Patera, W., & Bulling, A. (2014). Pupil: An open source platform for pervasive eye tracking and mobile gaze-based interaction. *UbiComp 2014 - Adjunct Proceedings of the 2014 ACM International Joint Conference on Pervasive and Ubiquitous Computing*, 1151–1160. https://doi.org/10.1145/2638728.2641695

Levatin, P. (1959). Pupillary Escape in Disease of the Retina or Optic Nerve. *A.M.A. Archives of Ophthalmology*, *62*(5), 768–779. https://doi.org/10.1001/archopht.1959.04220050030005

Litvan, I., Saposnik, G., Maurino, J., Gonzalez, L., Saizar, R., Sica, R. E. P., & Bartko, J. J. (2000). Clinical / Scientific Notes Selective sparing of pain pathways in a. *Neurology*, *54*(2). https://doi.org/10.1212/WNL.54.2.530

Loewenfeld, I. E. (1993). *The pupil: Anatomy, physiology and clinical applications*. Butterworth-Heinemann.

McDougal, D. H., & Gamlin, P. D. (2015). Autonomic control of the eye. *Comprehensive Physiology*, *5*(1), 439–473. https://doi.org/10.1002/cphy.c140014

Meeker, M., Du, R., Bacchetti, P., Privitera, C. M., Larson, M. D., Holland, M. C., & Manley, G. (2005). Pupil examination: validity and clinical utility of an automated pupillometer. *Journal of Neuroscience Nursing*, *37*(1), 34-40 7p.

Provencio, I., Rodriguez, I. R., Jiang, G., Hayes, W. P., Moreira, E. F., & Rollag, M. D. (2000). A novel human opsin in the inner retina. *Journal of Neuroscience*, *20*(2), 600–605. https://doi.org/10.1523/jneurosci.20-02-00600.2000

Spitschan, M. (2019). Melanopsin contributions to non-visual and visual function. *Current Opinion in Behavioral Sciences*, *30*(Figure 1), 67–72. https://doi.org/10.1016/j.cobeha.2019.06.004

Taylor, W. R., Chen, J. W., Meltzer, H., Gennarelli, T. A., Kelbch, C., Knowlton, S., Richardson, J., Lutch, M. J., Farin, A., Hults, K. N., & Marshall, L. F. (2003). Quantitative pupillometry, a new technology: Normative data and preliminary observations in patients with acute head injury - Technical note. *Journal of Neurosurgery*, *98*(1 SUPPL.), 205–213. https://doi.org/10.3171/jns.2003.98.1.0205

Thompson, H. S. (1966). Afferent pupillary defects. Pupillary findings associated with defects of the afferent arm of the pupillary light reflex arc. *American Journal of Ophthalmology*, *62*(5), 860–873. https://doi.org/10.1016/0002-9394(66)91911-8

Troiani, V. (2020). The future of quantitative pupillometry in health and disease. *Clinical Autonomic Research*, *0123456789*, 2–3. https://doi.org/10.1007/s10286-019-00655-3

Van Stavern, G. P., Bei, L., Shui, Y. B., Huecker, J., & Gordon, M. (2019). Pupillary light reaction in preclinical Alzheimer’s disease subjects compared with normal ageing controls. *British Journal of Ophthalmology*, *103*(7), 971–975. https://doi.org/10.1136/bjophthalmol-2018-312425

Winston, M., Zhou, A., Rand, C. M., Dunne, E. C., Warner, J. J., Volpe, L. J., Pigneri, B. A., Simon, D., Bielawiec, T., Gordon, S. C., Vitez, S. F., Charnay, A., Joza, S., Kelly, K., Panicker, C., Rizvydeen, S., Niewijk, G., Coleman, C., Scher, B. J., … Weese-Mayer, D. E. (2019). Pupillometry measures of autonomic nervous system regulation with advancing age in a healthy pediatric cohort. *Clinical Autonomic Research*. https://doi.org/10.1007/s10286-019-00639-3