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Supporting Data

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Chromatic Pupillometry in Isolated Rapid Eye Movement Sleep Behavior Disorder

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ABSTRACT: Background: Melanopsin retinal ganglion cell (mRGC)-mediated pupillary light reflex (PLR) abnormalities have been documented in several neurodegenerative disorders including Parkinson's disease. Overall, isolated rapid eye movement (REM) sleep behavior disorder (iRBD) represents the strongest prodromal risk factor for impending α-synucleinopathies.

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Objectives: To quantitatively compare PLR and mRGC-mediated contribution to PLR in 16 iRBD patients and 16 healthy controls.
Methods: iRBD and controls underwent extensive neuro-ophthalmological evaluation and chromatic pupillometry. In iRBD, PLR metrics were correlated with clinical variables and with additional biomarkers including REM atonia index (RAI), DaTscan, and presence of phosphorylated-α-synuclein (p-α-syn) deposition in skin biopsy.
Results: We documented higher baseline pupil diameter and decreased rod-transient PLR amplitude in iRBD patients compared to controls. PLR rod-contribution correlated with RAI. Moreover, only iRBD patients with evidence of p-α-syn deposition at skin biopsy showed reduced PLR amplitude compared to controls.
Conclusion: The observed PLR abnormalities in iRBD might be considered as potential biomarkers for the risk stratification of phenoconversion of the disease.

Key Words: pupillometry; REM sleep behavior disorder; synucleinopathy; neurodegeneration; melanopsin

Melanopsin retinal ganglion cells (mRGCs) are intrinsically photosensitive RGCs due to their expression of the photopigment melanopsin, modulating non-image forming functions of the eye as circadian photoentrainment and pupillary light reflex (PLR), via projections to the hypothalamic suprachiasmatic and olivary pretectal nuclei, respectively.[1] In vivo exploration of mRGC function is difficult since these cells represent a small RGC subgroup (about 1%) receiving inputs from rods and cones.[2]

Considering that mRGCs are maximally sensitive to blue light at 480 nm,[1,3] chromatic pupillometry protocols have been developed to isolate the mRGC contribution to the PLR[3] relying on light stimuli at different wavelengths.

Previous studies documented the presence of PLR abnormalities and mRGC dysfunction[4] as well as mRGC loss in post-mortem retinas of patients with Parkinson's disease (PD)[5] and altered pupil behavior has been recently described also in idiopathic/isolated rapid eye movement (REM) sleep behavior disorder.[6]

Idiopathic/isolated rapid eye movement (REM) sleep behavior disorder (iRBD) represents the strongest prodromal risk factor for α-synucleinopathies.[7] The presence of nigrostriatal dopaminergic denervation, motor symptoms, olfactory deficit, mild cognitive impairment, erectile dysfunction, color vision abnormalities, constipation, REM atonia loss, and age represent relevant biomarkers to predict the probability of phenoconversion.[7] Moreover, phosphorylated-α-synuclein (p-α-syn) deposits were detected in skin specimens of iRBD patients and proposed as diagnostic biomarker of underlying synucleinopathy.[8] P-α-syn deposits were also found in PD retinas.[9]

The aim of this study was to evaluate the PLR and mRGC-mediated contribution to PLR in iRBD.

Methods

This cross-sectional study included 16 iRBD patients consecutively diagnosed according to international diagnostic criteria[10] and 16 healthy controls. We received approval from the local ethical standards committee on human studies (EC Interaziendale Bologna-Imola #16032) and written informed consent was obtained from all patients participating in the study. Exclusion criteria were: spherical/cylindrical refractive errors more than 3 or 2 diopters; presence of posterior pole pathology including age-related macular degeneration and known optic neuropathies; ocular pressure more than 20 mmHg; severe lens opacity and/or retinal detachment and/or vascular retinal pathology; history of ophthalmologic surgery, except for uncomplicated cataract surgery, performed at least 6 months previously; shift-workers in the last year; and travels through more than one time zone during the last 3 months. Extensive neuro-ophthalmological evaluations were performed by using the protocol previously described.[3]

Controls underwent 7 days actigraphy (MotionWatch 8, CamNtech Ltd., Cambridge, UK) to ascertain absence of sleep disorders. Moreover, we computed the I < O index[11] and none of the controls displayed I < O values below the cut-off of 98.32.

Sleep questionnaires were administered (Epworth Sleepiness Scale [ESS]; Pittsburgh Sleep Quality Index, [PSQI]; Berlin questionnaire; REM sleep Behavior Disorder Screening Questionnaire [RBDSQ]) to evaluate the possible occurrence of sleep disturbances and RBD.

The whole group of iRBD patients was also investigated by means of history-taking looking for non-motor and motor symptoms, neurologic examination including evaluation with the Unified Parkinson's Disease Rating Scale Part III (UPDRS-III), extensive neuropsychological tests, brain magnetic resonance imaging (MRI), and nigrostriatal dopamine transporter ligand [^{123}I]-ioflupane single-photon emission computed tomography (SPECT) (DaTscan). All iRBD patients underwent nocturnal polysomnography and REM Atonia Index (RAI) was computed, using the validated automatic analysis implemented in Hypnolab v. 1.2 software.[12] For all patients we also retrieved data of skin biopsy for p-α-syn deposits by obtaining in total eight 3 mm punches biopsies for each patients (ie, two at the cervical C8 level left and right and two at the distal leg left and right), which were then analyzed according to previously reported methods.[13]

The chromatic pupillometry protocol is described in detail elsewhere.[3] A Ganzfeld ColorDome full-field stimulator (Espion V6, ColorDome Desktop Ganzfeld;

Diagnosys LLC; Lowell, MA, USA) was used. Participants were dark-adapted for 10 minutes, and colored light stimuli were presented to the tested eye and the pupil responses were recorded from the same eye using the Ganzfeld system equipped with an integrated pupillometer. Stimuli consisted of short wavelength (blue flash, mid = 472 nm) and long wavelength (red flash, mid = 632 nm) light flashes of 1 second duration. The integrated pupillometer system measured the pupil diameter at a 100 Hz sampling frequency. The inter-stimulus interval (ISI) was 20 seconds for the rod- and cone- conditions (for both red and blue stimuli), while for the mRGC-condition ISI was 30 seconds for red stimulus and 70 seconds for the blue one. The light conditions used were the following:

- rod-condition: low luminance blue flash (0.001 cd/m^2, 472 nm) under dark-adaption;
- mRGC-condition: high luminance blue flash (450 cd/m^2, 472 nm) under dark-adaption;
- cone-condition: red flash (10 cd/m^2, 632 nm) presented against 6 cd/m^2 rod-suppressing blue-adapting field.

Data were analyzed using custom MATLAB scripts (MathWorks Inc., Natick, MA, USA)[3] and the calculated pupillometric parameters were transient PLR amplitude, defined as the difference between the normalized baseline and the minimum normalized PLR after stimulus onset (pupil maximum constriction), and post-illumination pupil response (PIPR) for evaluating the mRGC-sustained response, defined as the difference between the normalized baseline and the normalized median PLR measured over a 5–7 second time interval from stimulus offset.

Statistical data analysis included Chi-square, Wilcoxon signed-rank tests, and Spearman correlation coefficient analysis to measure the association between pupillometric parameters and RAI in iRBD. We also stratified iRBD patients according to the presence or absence of p-α-syn deposition in skin biopsy and carried out Kruskal-Wallis H test followed by Dunn-Bonferroni post hoc to compare PLR metrics between iRBD (p-α-$syn_{presence}$, n = 12; p-α-$syn_{absence}$, n = 4) subgroups and controls (n = 16). For optical coherence tomography (OCT) data, we evaluated eyes tested by pupillometry.

Statistical analyses were performed using SPSS, version 20.0 (SPSS Inc., IBM, Chicago, IL, USA) software.

Results

Table 1 shows the demographics and clinical characteristics of the two groups. Controls and iRBD patients did not significantly differ in terms of age ($P = 0.29$) and gender distribution ($P = 0.15$).

Raw pupil traces showed excessive blink artifacts under rod-, mRGC-, and cone-conditions in one, two, and three controls respectively, and in one iRBD patient under the cone-condition and thus were removed from data analysis.

For rod- (Fig. 1A,B) and cone- (Fig. 1E,F) conditions, PLR is characterized by a rapid transient constriction followed by a relatively rapid return to baseline (Fig. 1A, E, B,F). Under mRGC-condition, the PLR is characterized by an initial transient constriction followed by a sustained constriction (PIPR) (Fig. 1C,D).

Baseline normalized pupil size was significantly larger in iRBD patients compared to controls in rod- ($P = 0.027$) and mRGC- ($P = 0.01$) conditions (Table 1). Rod-transient PLR amplitude was significantly decreased ($P = 0.024$) in iRBD patients compared to controls, while mRGC-mediated PIPR did not differ between groups (Table 1). Transient PLR amplitude was not significantly different between iRBD patients and controls under the cone-condition (Table 1).

In iRBD patients there were no significant differences in rod-transient PLR amplitude and PIPR among subgroups with/without autonomic symptoms, hyposmia, and DaTscan abnormalities (data not shown). Furthermore, we found a significant correlation between RAI and rod-transient PLR amplitude (r = 0.55, $P = 0.03$).

For iRBD patients we evaluated the presence of p-α-syn deposition in skin biopsy (p-α-$syn_{presence}$: n = 12, 75%; p-α-$syn_{absence}$: n = 4, 25%; Table S1). A significant difference between iRBD patients with p-α-syn deposition in skin biopsy and controls was evident for the rod-transient PLR amplitude (controls, median [Q1-Q3] = 0.28 [0.22–0.35]; p-a-$syn_{presence}$, median [Q1-Q3] = 0.19 [0.12–0.27]; $p_{adjusted} = 0.043$) and the baseline normalized pupil size under rod- (controls, median [Q1-Q3] = 5.8 [5.0–6.6]; p-a-$syn_{presence}$, median [Q1-Q3] = 7.1 [6.6–7.9]; $p_{adjusted} = 0.026$) and mRGC- (controls, median [Q1-Q3] = 5.5 [4.6–5.9], $syn_{presence}$, median [Q1-Q3] = 6.7 [6.2–7.2]; $p_{adjusted} = 0.019$) conditions.

Mean comparisons of OCT measurements for peripapillary Retinal Nerve Fiber Layer (pRNFL) quadrants and macular ganglion cell layer (GCL) + sectors did not show significant differences between iRBD patients and controls (data not shown).

Discussion

Our exploratory study shows that PLR rod-contribution is impaired in iRBD and correlates with RAI, which is a diagnostic and prognostic marker of iRBD. Interestingly, a significant reduction of PLR amplitude in the rod-condition compared to controls was evident only for iRBD subjects with a documented underlying synucleinopathy, as demonstrated by the findings of positive skin biopsy for p-α-syn deposits in the skin.

TABLE 1 *Sociodemographic/clinical characteristics and pupillometric parameters in controls and idiopathic/isolated rapid eye movement (REM) sleep behavior disorder (iRBD) patients*

Parameter	Controls	iRBD patients	*P* value
Number	16 (50%)	16 (50%)	
Gender			
Male	7 (43.8%)	12 (75%)	0.15
Female	9 (56.2%)	4 (25%)	
Age, years	66.5 (62.25–70.25)	70.5 (63.5–73.0)	0.29
Age class, years			
50–59	2 (12.5%)	2 (12.5%)	0.63
60–69	9 (56.2%)	6 (37.5%)	
70–79	5 (31.2%)	8 (50%)	
Disease duration	NA	9.2 ± 6.8	NA
Autonomic dysfunction	NA		NA
Constipation		4/16 (26.7%)	
Urinary symptoms		4/16 (26.7%)	
Orthostatic hypotension		2/16 (12.5%)	
Hyposmia	NA	8/16 (53.3%)	NA
Dopamine imaging[a]	NA	4/16 (26.7%)	NA
Cognitive evaluation	Normal	Normal	
ROD			
Number	15	16	
Baseline normalized pupil size (0.001 cd/m^2, 472 nm)			
Median (Q1-Q3)	5.74 (5.03–6.56)	7.14 (5.64–7.80)	0.027
Transient peak amplitude (0.001 cd/m^2, 472 nm)			
Median (Q1-Q3)	0.29 (0.22–0.35)	0.23 (0.12–0.27)	0.024
MELANOPSIN			
Number	14	16	
Baseline normalized pupil size (450 cd/m^2, 472 nm)			
Median (Q1-Q3)	5.47 (4.64–5.95)	6.81 (5.94–7.19)	0.01
PIPR (450 cd/m^2, 472 nm)			
Median (Q1-Q3)	0.48 (0.45–0.52)	0.50 (0.45–0.53)	0.79
CONE			
Number	13	15	
Baseline normalized pupil size (10 cd/m^2, 632 nm)			
Median (Q1-Q3)	3.19 (2.98–3.87)	3.76 (3.45–4.07)	0.17
Transient peak amplitude (10 cd/m^2, 632 nm)			
Median (Q1-Q3)	0.24 (0.20–0.28)	0.20 (0.15–0.27)	0.22

Values are given as number (absolute frequency) or median (Q1-Q3, interquartile range); Chi-square test was performed with categorical variables and Wilcoxon signed-rank test was used with continuous variables.

[a]Reduced nigrostriatal dopaminergic binding assessed by [123I]-ioflupane single-photon emission computed tomography (SPECT) (DaTscan).

Abbreviations: NA, not applicable; iRBD, idiopathic/isolated rapid eye movement (REM) sleep behavior disorder; PIPR, post-illumination pupil response.

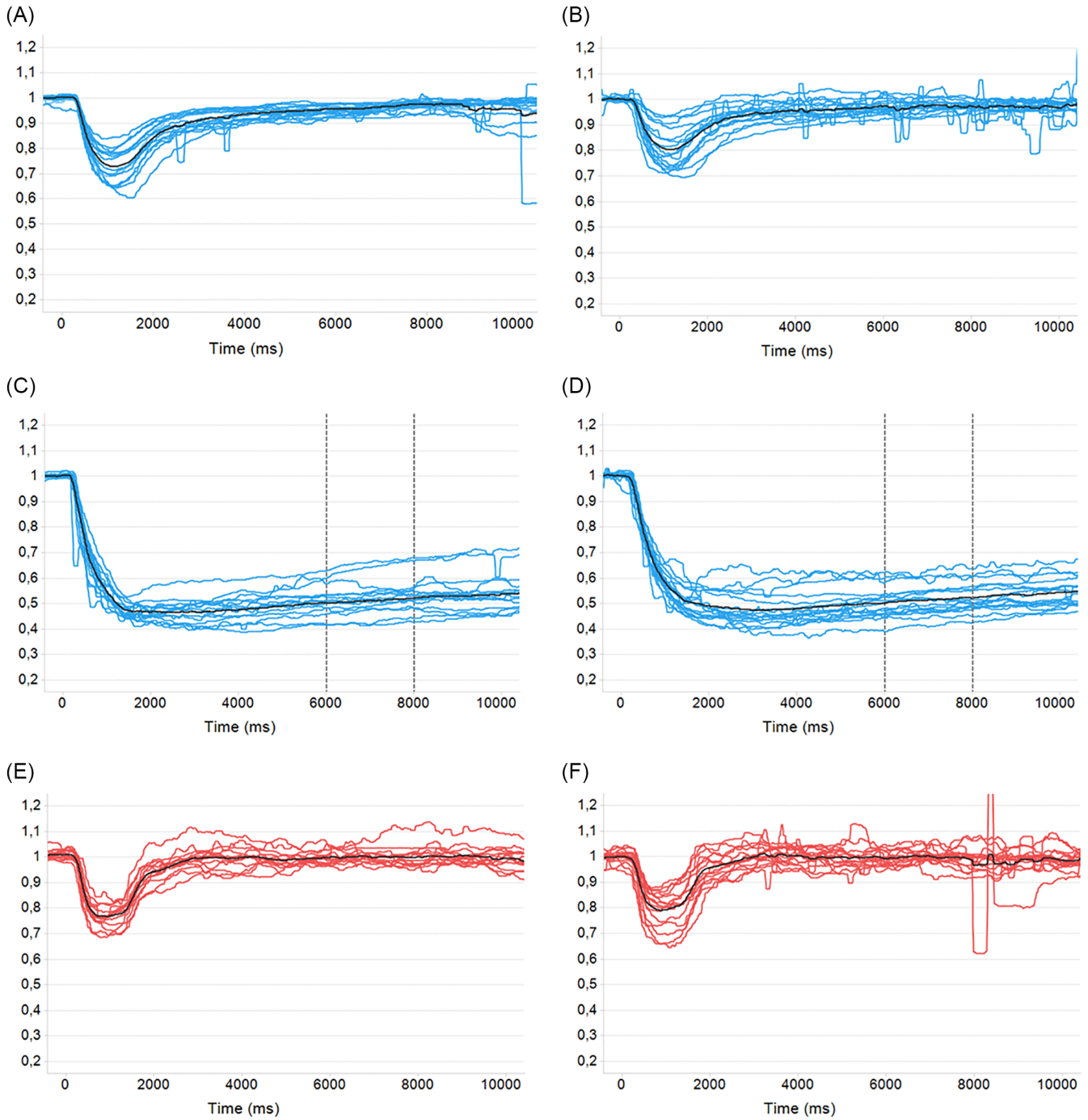

FIG 1. Single and mean normalized pupillary light reflex (PLR) curves for the rod-, melanopsin retinal ganglion cell (mRGC)-, and cone- conditions in controls and idiopathic/isolated rapid eye movement (REM) sleep behavior disorder (iRBD) patients. Pupillometric traces obtained under the rod- (**A** and **B**), mRGC- (**C** and **D**), and cone- (**E** and **F**) conditions of the chromatic pupillometric protocol. Light blue (**A**, **B**, **C**, **D** panels) and red (**E**, **F** panels) traces represent single individuals, while black traces (**A**–**F** panels) represent the mean waveforms for each group (**A**, **C**, **E** for the control group; **B**, **D**, **F** for the iRBD group). The vertical dotted lines indicate the time range (5–7 seconds from stimulus offset) over which the mRGC-mediated (sustained) amplitude (post-illumination pupil response [PIPR], 450 cd/m^2) was measured. [Color figure can be viewed at wileyonlinelibrary.com]

The higher baseline pupil diameter and reduced rod-transient amplitude in iRBD unravels a likely parasympathetic dysfunction. This observation is in line with the cholinergic deficiency already reported by previous pupillometry studies in PD.[14]

Moreover, the presence of rod-mediated transient PLR amplitude abnormalities may putatively reflect a pathology affecting mRGC dendrites before hitting the mRGC cell body,[3] as supported by the absence of PIPR reduction in our cohort of iRBD subjects. The

significantly reduced transient peak amplitude in conditions exploring the rod-contribution may, in fact suggest an altered contact between rods and mRGCs. mRGCs receive synaptic input from rods and cones through bipolar cells and a direct contact of rod bipolar cells via ribbon synapses in the ON layer of the inner plexiform layer (IPL) with mRGC dendrites has been demonstrated in human retinas.[2] It is plausible to hypothesize that the rod response depends more on the distal dendrites of mRGCs, and consequently that the subsequent reduced dendritic arborization might interfere with the rod input out of proportion to the cones. The occurrence of mRGC dendropathy has been already reported in PD,[5] as well as a specific affection of the rod pathway through the dopaminergic amacrine cells (AII) synaptically contacting mRGCs.[15]

The association of PLR abnormalities with two of the most powerful markers of impending neurodegeneration in iRBD strengthens our results. Indeed, skin biopsy has consistently demonstrated great sensitivity and specificity as an in vivo diagnostic biomarker of an underlying synucleinopathy in iRBD.[13,16] Similarly, REM sleep without atonia has been identified as one of earliest signs of neurodegeneration[17,18] and a valuable prognostic biomarker of disease progression, as it increases over time and greater severity is associated with accelerated phenoconversion.[19,20]

One study limitation is the small sample size. However, relying on our current results, chromatic pupillometry abnormalities might be proposed as a further potential early marker of parasympathetic affection and/or of mRGC dysfunction in iRBD. Longitudinal studies using larger cohorts are needed to validate pupillometry metrics as an adjunctive biomarker and also for the risk stratification of phenoconversion in iRBD. ●

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Data

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