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Pedro Lax, Oksana Kutsyr, Gema Esquiva, Cesare Altavilla, Victoria Maneu, Nicolás Cuenca

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Title: Cannabinoid-mediated retinal rescue correlates with improved circadian parameters in retinal dystrophic rats

Author names and affiliations: Pedro Lax^a, Oksana Kutsyr^a, Gema Esquiva^b, Cesare Altavilla^a, Victoria Maneu^b and Nicolás Cuenca^{a,c}

^aPhysiology, Genetics and Microbiology, University of Alicante, Alicante, Spain.

^bOptics, Pharmacology and Anatomy, University of Alicante, Alicante, Spain.

^cInstitute Ramón Margalef, University of Alicante, Alicante, Spain.

Running title: Retinal rescue correlates to circadian improvement

Corresponding author: Pedro Lax, Department of Physiology, Genetics and Microbiology, University of Alicante, Carretera San Vicente del Raspeig, 03690 San Vicente del Raspeig, Alicante, Spain. pedro.lax@ua.es, phone: +34 96590 3856, fax: +34 96590 3943.

ABSTRACT

Ocular pathologies and blindness have been linked to circadian disorders. In previous studies, our group has demonstrated that retinitis pigmentosa is associated with degenerative changes in the melanopsin system and weaker circadian patterns. We have also shown that cannabinoids preserve retinal structure and function in dystrophic P23H rats. This study is consequently aimed at examining whether the morphologic and functional rescue of retinal degeneration by cannabinoids is associated with amelioration of circadian parameters. The synthetic cannabinoid HU210 (100 μ g/kg, i.p.) or vehicle were administered to transgenic P23H rats three times per week, from postnatal day 24 to 90. Sprague-Dawley rats were used as a healthy control group. Locomotor activity and scotopic electroretinograms were recorded, and the retinal structure was analyzed at the end of the experiment. The ERG a- and b-wave amplitudes and photoreceptor cell number were more deteriorated in vehicle-administered P23H rats as compared to P23H rats treated with HU210. In cannabinoid-administered P23H rats, the locomotor activity circadian rhythms showed less disturbance than that observed in vehicle-administered P23H rats, the latter showing lower values for mesor, amplitude, acrophase, percentage of variance and non-parametric variables. A positive linear correlation was found between retinal values and circadian parameters of locomotor activity from P23H rats. This study thus provides evidence of a positive correlation between cannabinoid-mediated rescue of retinal structure and function and improvement of circadian rhythmicity.

Key Words: Retinal degeneration; retinitis pigmentosa; P23H; HU210; circadian rhythms

Abbreviations: CB1 and CB2, cannabinoid receptor type 1 and 2; CFI, circadian function index; ERG, electroretinogram; IS, interdaily phase stability; IV, intradaily variability; LA, locomotor activity; LD, light/dark; mRGCs, melanopsin-containing retinal ganglion cells; ONL, outer nuclear layer; PB, phosphate buffer; RA, relative amplitude; RP, retinitis pigmentosa; SD, Sprague-Dawley; THC, Δ^9 -tetrahydrocannabinol

1. Introduction

Light is the most common and foremost zeitgeber for circadian rhythms (Aschoff, 1960, 1963). In mammals, light synchronization occurs through direct input from melanopsin-containing retinal ganglion cells (mRGCs), which are intrinsically photosensitive (Berson et al., 2002; Freedman et al., 1999; Gooley et al., 2003; Hattar et al., 2002; Hattar et al., 2003; Lucas et al., 2001; Lucas et al., 2003; Panda et al., 2003; Panda et al., 2002; Ruby et al., 2002). mRGCs require no synaptic inputs to drive circadian photoentrainment (Foster et al., 1991; Lucas et al., 2001; Provencio et al., 1994), but they receive these inputs from amacrine and bipolar cells (Belenky et al., 2003; Vugler et al., 2015). Accordingly, melanopsin is not required in order for the circadian clock to receive photic information; melanopsin knockout mice (*Opn4*^{-/-}) still retain circadian photoentrainment (Panda et al., 2002; Ruby et al., 2002), and rod photoreceptors are capable of driving photic entrainment of the circadian rhythms of animals in which the only functional photoreceptors are rods (Altimus et al., 2010). Thus, rod-cone photoreceptors and mRGCs signal circadian photoentrainment in a complementary manner (Altimus et al., 2010; Guler et al., 2008), and the complete loss of circadian oscillator photoentrainment requires a deficiency of both inner and outer retina photoreceptors (Panda et al., 2003). Hence, in ocular pathologies and blindness, circadian disorders exhibit a direct relationship to the degree to which light perception is preserved (de Zavalía et al., 2011; Gonzalez Fleitas et al., 2015; Lahouaoui et al., 2014; Lockley et al., 2007; Skene and Arendt, 2007).

In the retina, evidence has been reported supporting cannabinoid modulation of photoreceptor cells (Fan and Yazulla, 2003; Straiker and Sullivan, 2003), bipolar cells (Straiker

et al., 1999; Yazulla et al., 2000) and ganglion cells (Lalonde et al., 2006; Middleton and Protti, 2011), and neuroprotective actions of cannabinoids have been reported in excitotoxic damage (El-Remessy et al., 2003; Kokona and Thermos, 2015; Opere et al., 2006), ischemia (Araujo et al., 2017; Nucci et al., 2007; Pinar-Sueiro et al., 2013) and glaucoma (Cairns et al., 2016; Nucci et al., 2008; Rapino et al., 2017). In a previous study, we demonstrated that systemic administration of the synthetic cannabinoid HU210 slows retinal neurodegeneration in the P23H rat model of autosomal dominant retinitis pigmentosa (Lax et al., 2014).

Considering the profound impact of vision on the functioning of the mammalian circadian timing system, it is important to assess a possible link between rescue of retinal degeneration and improvement of circadian rhythm disruptions. The purpose of this study is therefore to assess the relationship between changes in retinal morphology or function and changes in circadian parameters in dystrophic P23H rats treated or untreated with the cannabinoid agonist HU210. A positive correlation between the effect on both the circadian and visual systems may reinforce the potential use of cannabinoids in preventing RP.

2. Materials and methods

2.1. Animals and housing conditions

P23H line 3 homozygous male albino rats ($n = 11$), obtained from Dr. M. LaVail (UCSF), were used as an animal model of RP. Male Sprague-Dawley (Ryan et al.) rats (genetic background; $n = 5$) (Harlan Laboratories, Barcelona, Spain) were used as a healthy control group. Animals were all born in the animal colony at the University of Alicante and housed in controlled photoperiod (LD 12:12), humidity (55-60%) and temperature (23 ± 1 °C) conditions. Food and water were both provided *ad libitum*. Procedures involving animal experimentation had been previously approved by the ethics committee of the University of Alicante (Reference: UA-07/22/2013). The animals were handled according the rules governing the care and use of laboratory animals in order to minimize animal suffering and reduce the number of animals used per experiment (European Directive 2010/63/UE).

2.2. Experimental design

The synthetic cannabinoid HU210 was obtained from Tocris Bioscience (Tocris, Bristol, UK). A total of six P23H rats were administered with the cannabinoid ($100 \mu\text{g/kg}$, i.p.) three times per week, from postnatal day 24 to 90 (P23H + HU210 group), while five P23H rats received the same volume of sterile saline at each time point (P23H group). The body weight of the animals was measured prior to each injection in order to calculate the dose of HU210 or vehicle. Healthy SD rats were not administered any substance (SD group).

2.3. Locomotor activity recording

The last nine days of the experiment (P81 to P90), spontaneous locomotor activity (LA) was collected continuously over 10 min intervals by means of an infrared photocell (OMRON, E3S-AD62, Japan) installed in each animal cage. A computer detected and collected data signals for subsequent analysis. Locomotor activity was expressed according to activity counts, specifically, the mean number of photocell counts per 10-min period.

LA records were analyzed using software designed for analysis of chronobiological data (El temps; Diez-Noguera, University of Barcelona). We obtained actograms, mean waveforms, cosinor and Sokolove-Bushell periodograms. In addition, a non-parametric analysis of the data was also carried out (Lax et al., 2012; Lax et al., 2011). The interdaily phase stability (*IS*) values (ranging from 0 to 1) represent a measure of the rhythm phase stability over consecutive days and quantify the synchronization to the 24-h LD cycle. The intradaily variability (*IV*), which ranges from 0 to 2, expresses the degree of rhythm fragmentation. The relative amplitude (*RA*) determines the ratio between the most active 10-hour period and the least active 5-hour period. The circadian function index (*CFI*) is an integrated parameter; it incorporates *RA*, *IS* and *IV* values.

2.4. Electrorretinography

Scotopic electroretinograms (ERG) were performed at P90 after overnight adaptation to the dark. Animals were anesthetized under dim red light with an i.p. injection of a mixture of ketamine (100 mg/kg) and xylazine (4 mg/kg), and placed on a heating pad at 38 °C. A topical application of 1% tropicamide (Alcon Cusí, Barcelona, Spain) was used to dilate the pupils. 0.2% polyacrylic acid carbomer (Viscotears; Novartis, Barcelona, Spain) was applied to the

cornea to prevent dehydration and allow for electrical contact with the recording electrodes (DTL; Sauquoit Industries, Scranton, PA, USA). A 25-gauge platinum needle inserted under the scalp between the eyes served as reference electrode. The ground connection was a gold electrode inserted in the mouth. 10 ms light pulses were administered to the animals at eleven increasing luminances, at intervals of 10 s for dim flashes (up to $-1.4 \log \text{cd} \cdot \text{s/m}^2$) and 20 s for the brightest flashes (from $-0.8 \log \text{cd} \cdot \text{s/m}^2$). ERG recordings were amplified and band-pass filtered (1-1000 Hz, without notch filtering) using a data acquisition board (DAM50; World Precision Instruments, Aston, UK). Stimulus generation and data acquisition (4 kHz) were performed with digital acquisition system (PowerLab System; AD Instruments, Oxfordshire, UK). The amplitude of the a-wave was measured from the prestimulus baseline to the most negative trough of the ERG, and that of the b-wave from the trough of the a-wave to the peak of the b-wave.

2.5. Retinal Histology

Histological studies were conducted at P90, after ERG recording. Vertical cryostat sections were prepared, according to procedures that are well-established in the literature (Fernandez-Sanchez et al., 2017; Noailles et al., 2016). Animals were sacrificed in the morning with a lethal dose of pentobarbital sodium. The dorsal limbus of each eye was identified with a stitch, after which the eyes were enucleated, fixed in 4% (w/v) paraformaldehyde for 1 h, washed in 0.1 M phosphate buffer pH 7.4 (PB) and cryoprotected in 15, 20 and 30% sucrose. The cornea, lens and vitreous body were extracted, and the eyecups were embedded in OCT media (Tissue-Tek; Sakura Finetek, Zoeterwouden, Netherlands) and frozen in liquid nitrogen. Sections with a

thickness of 16 μm were then obtained at $-25\text{ }^{\circ}\text{C}$ in a cryostat (CM 1900; Leica Microsystems, Wetzlar, Germany), mounted on microscope slides (Superfrost Plus; Menzel GmbH & Co KG, Braunschweig, Germany) and air-dried.

The thickness of the outer nuclear layer (ONL) was measured and used as an indicator of photoreceptor number. For measurements of ONL thickness, a nuclear stain (TO-PRO-3; Molecular Probes, Eugene, OR, USA) was added at 1 $\mu\text{g}/\text{ml}$ to at least 3 sections per animal that included the optic nerve and the temporal and nasal ora serrata. Images were processed with a standard image processing software (Adobe Photoshop 10; Adobe Systems Inc., San Jose, CA, USA). Measures were performed every 0.5 mm from the optic nerve to the ora serrata. All quantifications were performed in a blinded manner by two independent observers.

2.6. Statistical analysis

Statistical analyses were done using SPSS software (SPSS, Chicago, IL, USA). A two-way ANOVA was performed to assess the effects of genotype (Sprague-Dawley *vs.* P23H) and treatment (HU210 *vs.* vehicle), and any interactions among them. A Bonferroni's test was used for post hoc pairwise comparisons when the level of significance was 0.05. All analyzed categories showed normal distributions and variance homogeneity. A Student's *t* test was used in cases where only two groups were compared. A regression analysis was carried out to determine the relationship between circadian parameters and retinal function (maximum scotopic b-wave amplitudes) or structure (ONL thickness). A *p* value of < 0.05 were considered statistically significant. Data were plotted as the mean $\pm$ standard error of the mean.

3. Results

3.1. Cannabinoids reduce retinal function deterioration

Single-flash ERGs were obtained from normal SD rats ($n = 5$) and dystrophic P23H rats treated or untreated with the cannabinoid HU210 ($n = 6$ and $n = 5$, respectively). As can be seen in Fig. 1, ERG responsiveness suffered greater deterioration in vehicle-administered P23H rats than in those treated with HU210 (postnatal day 24 to 90), as compared to that observed in healthy SD rats. The mean amplitudes for a- and b-waves recorded under scotopic conditions at P90 were larger in HU210-treated P23H rats than that observed in vehicle-administered transgenic animals (ANOVA, Bonferroni's test, Fig. 1B). At this age, HU210-treated P23H rats showed maximum recorded scotopic a- (a-max) and b-waves (b-max) that were around 80% and 70% higher, respectively, than those observed in vehicle-administered dystrophic animals.

3.2. Cannabinoids reduce photoreceptor cells death

The protective effect of HU210 on photoreceptor cells was assessed by measuring the thickness of the ONL. Fig. 2 shows cross-sections of P90 retinas from a SD rat (Fig. 2A), a P23H rat administered the vehicle (Fig. 2B) and a P23H rat treated with HU210 (Fig. 2C). Fewer photoreceptor nuclei were seen in the ONL of the vehicle-treated P23H rats versus healthy SD rats. Nevertheless, despite the evident thinning of the retina of HU210-treated rats, their ONL were thicker than what was observed in vehicle-administered transgenic animals. Since the degeneration in the retinas of P23H rats was not homogeneous across the entire retina, we quantified the ONL thickness in eighteen different retinal locations, from temporal to nasal positions. The thickness of the ONL was determined to be higher in HU210-treated P23H rats

($n = 5$) than in vehicle-administered dystrophic animals ($n = 5$) in all examined locations, with significant differences at the temporal ora serrata and in the nasal area of the retina, at 1.5 and 2 mm from the optic nerve (ANOVA, Bonferroni's test, Fig. 2D). On average, the mean ONL thickness in HU210-treated P23H rats was 28% greater than that observed in P23H rats administered the vehicle ($13.0 \pm 0. \mu\text{m}$ vs. $10.1 \pm 0.9 \mu\text{m}$; Fig. 2D).

3.3. Circadian rhythmicity of locomotor activity improves in P23H rats treated with cannabinoids

To assess circadian rhythms and photoentrainment in dystrophic P23H rats treated or untreated with the synthetic cannabinoid HU210 and compare it to observations in Sprague-Dawley rats, the locomotor activity of each animal was continuously monitored throughout the last nine days of the experiment (P81 to P90). Fig. 3 shows representative locomotor activity actograms, Sokolove-Bushell periodograms and mean waveforms for a SD rat (Fig. 3A), a P23H rat administered the vehicle (Fig. 3B) and a P23H rat treated with HU210 (Fig. 3C), all of which were entrained to a 12:12 LD cycle. Locomotor activity demonstrated a robust daily rhythm in all animals, regardless of the strain of the animal or treatment. However, we observed a slight deterioration in the circadian locomotor activity rhythms of vehicle-administered P23H rats (Fig. 3B), with weak circadian oscillations in actograms and mean waveforms and lower significance of peaks in the periodograms. In these animals, the amplitude and the percent of variance accounted for by the locomotor activity rhythms were significantly smaller than those observed in SD rats (Student's t test, $p < 0.05$ and $p < 0.01$, respectively; Table 1). In P23H rats treated with the cannabinoid, the circadian rhythm parameters evaluated were similar to those

observed in SD rats, with mesor, amplitude, acrophase and percentage of variance values greater than those of vehicle-administered P23H rats (Student's t test; Table 1).

To understand the effects of chronic cannabinoid treatment on locomotor activity rhythms of P23H rats, the degree of phase homogeneity in each animal was evaluated by means of non-parametric variables. This analysis also evidenced that locomotor activity circadian rhythms were less robust in vehicle-administered P23H rats, as compared to the SD animals. The strength of the coupling of the locomotor activity rhythms to environmental zeitgebers (IS) and the relative amplitude of the rhythm (RA) were significantly lower in P23H rats versus SD rats (Student's t test, $p < 0.05$ in both cases; Table 1). Meanwhile, locomotor activity records in HU210-treated P23H rats showed non-parametric values similar to those observed in SD rats, with interdaily stability, relative amplitude and circadian function index values significantly greater than those seen in vehicle-administered P23H rats. Unexpectedly, intradaily variability values were significantly lower in vehicle-administered P23H rats as compared to both healthy SD rats and HU210-treated P23H rats (Student's t test, $p < 0.01$ and $p < 0.05$, respectively; Table 1).

3.4. Retinal and circadian parameters correlate in P23H rats treated or untreated with cannabinoids

To assess a potential relationship between cannabinoid-mediated rescue of retinal degeneration and amelioration of circadian rhythm disturbances, we correlated morphological and functional values with parametric and non-parametric scores from locomotor activity circadian rhythms in dystrophic P23H rats treated or untreated with the cannabinoid HU210. A

positive linear correlation was found between retinal values and circadian parameters of locomotor activity in P23H rats. Specifically, the maximum recorded b-wave amplitudes obtained in vehicle- and HU210-administered P23H rats significantly correlated with the amplitude ($R = 0.66$), acrophase ($R = 0.71$), interdaily stability ($R = 0.61$) and relative amplitude ($R = 0.81$) of the locomotor activity circadian rhythms of these animals (Fig. 4A, Table 1). Similarly, ONL thickness values significantly correlated with the mesor ($R = 0.65$), amplitude ($R = 0.63$), acrophase ($R = 0.79$), interdaily stability ($R = 0.74$) and relative amplitude ($R = 0.67$) of locomotor activity circadian rhythms (Fig. 4B, Table 1). Intradaily variability values showed an unexpected positive correlation with respect to both ONL thickness and b-wave amplitudes ($R = 0.69$ and $R = 0.71$, respectively).

4. Discussion

Results of this study provide evidence that retinitis pigmentosa is linked to both a progressive degeneration of retinal structure and function and a gradual deterioration of circadian rhythms, and that chronic administration of cannabinoids is able to rescue both retinal degeneration and circadian disturbances. Previous studies have extensively shown that ocular pathologies are associated with circadian rhythm disturbances (Cugini et al., 2001; Lucas et al., 2003; Lupi et al., 1999; Mrosovsky et al., 1999; Panda et al., 2003; Panda et al., 2002), and our previous studies have demonstrated that melatonin administration partially reverses both vision loss and circadian rhythm fragmentation in dystrophic rats (Lax et al., 2011), but to our knowledge this is the first study to show a positive correlation between rescue of retinal structure and function and improvement of circadian rhythms.

The present study concerns P23H rats, which have been engineered to mimic a naturally occurring rhodopsin mutation in P23H patients (Cuenca et al., 2004; Machida et al., 2000; Pinilla et al., 2005). In both dystrophic and control animals, circadian rhythms parameters were obtained by recording locomotor activity. Previous studies conducted by our research group in this experimental model indicate that circadian parameters obtained from locomotor activity recordings are comparable to those obtained from other variables such as body temperature (Lax et al., 2016; Lax et al., 2011). In our results, the average locomotor activity in dystrophic animals was similar to that observed in control animals, indicating that, under our experimental conditions, vision impairment does not affect locomotor activity.

The protective effects of synthetic cannabinoid HU210 on the retina of dystrophic P23H rats agree with a previous study of our group demonstrating that cannabinoids preserve the structure and function of the retina in P23H rats (Lax et al., 2014), and with other earlier studies in which we assessed the neuroprotective effects of antioxidant and antiapoptotic agents (Fernandez-Sanchez et al., 2017; Fernandez-Sanchez et al., 2012a; Fernandez-Sanchez et al., 2012b; Fernandez-Sanchez et al., 2015; Fernandez-Sanchez et al., 2011; Roche et al., 2016). These neuroprotective actions of HU210 might be mediated by stimulation of the endocannabinoid system. Immunoreactivity for cannabinoid receptor type 1 (CB1) and type 2 (CB2) has been found in the retinas of rodents and primates (Bouskila et al., 2012; Bouskila et al., 2013; Lopez et al., 2011; Straiker et al., 1999; Yazulla, 2008; Yazulla et al., 1999). HU210 has a high affinity for both receptors (Pertwee, 1999), and has been shown to be 100 to 800 times more potent than the main psychoactive constituent of marijuana, Δ^9 -tetrahydrocannabinol (THC) (Ottani and Giuliani, 2001).

With respect to the effects of the cannabinoid on circadian patterns, there are some reports suggesting that cannabinoids may directly affect circadian rhythms. CB1 receptors are expressed in the suprachiasmatic nuclei (Acuna-Goycolea et al., 2010; Wittmann et al., 2007) of mice, and the presence of CB1 and CB2 receptor proteins has been demonstrated in the rat pineal gland (Koch et al., 2008). Besides, the endocannabinoid system has been shown to be capable of modulating the rat sleep-waking cycle (Santucci et al., 1996) and circadian rhythms in hamsters (Sanford et al., 2008). Furthermore, endocannabinoids exert time-dependent control in the pineal gland (Koch et al., 2015). Exogenous administration of cannabinoids has no effect on the endogenous free-running circadian rhythm, however, it does reduce the capacity of the circadian clock to entrain to light zeitgebers (Acuna-Goycolea et al., 2010).

In our results, chronic exogenous administration of the synthetic cannabinoid HU210 reduced the disturbances in the locomotor activity circadian patterns of dystrophic P23H rats, with differences in parametric and non-parametric variables as compared to the observations in untreated transgenic animals. This result agrees with a previous study from our group in which chronic melatonin administration to P23H rats was found to decrease vision loss and circadian rhythmicity impairment in P23H rats in dystrophic animals (Lax et al., 2011). In this previous study, melatonin-mediated improvements in the circadian patterns correlated with melatonin-dependent amelioration of the visual function, similarly to the correlation we have observed here between the rescue of retinal structure and function and improvement of circadian rhythms.

5. Conclusion

In light of the evidence presented here, it can be concluded that the administration of exogenous cannabinoids decreases circadian rhythmicity impairment and vision loss in P23H rats. This would point to its further potential use in the clinical management of retinitis pigmentosa, as well as the possible disorders associated with vision loss, such as circadian disruption and the subsequent development and maintenance of mood disorders. Further studies are required, however, in order to provide more information on the mechanism behind these effects.

Declaration of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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Figure legends

Fig. 1. ERG responsiveness in SD rats and in P23H rats treated or untreated with HU210. (A) Representative scotopic ERG traces at P90 from a SD rat (left), a control P23H rat (middle) and a P23H rat treated with HU210 (right). (B) Luminance-response curves of SD rats (circles) and P23H rats administered vehicle (squares) or HU210 (diamonds), revealing statistically significant a- and b-wave amplitude (left and right, respectively) differences between both treatments. $N = 5-6$ rats per group, $n = 3-10$ recordings per rat. Results are presented as mean $\pm$ SEM. Two-way ANOVA, Bonferroni post-hoc test, $*p < 0.05$, $**p < 0.01$.

Fig. 2. Photoreceptor number in SD rats and in P23H rats treated or untreated with HU210. (A-C) Vertical sections of P90 retinas from a SD rat (A), a control P23H rat (B) and a P23H rat treated with HU210 (C), stained with the nuclear marker TO-PRO to visualize retinal cells. Images taken in the nasal area, 0.5 mm away from the optic nerve. (D) Quantification of ONL thickness along sections of central retina from the temporal to the nasal ora serrata in SD rats (circles) and P23H rats administered the vehicle (squares) or HU210 (diamonds), showing statistically significant differences between treatments. $N = 5$ rats per group, $n = 3$ sections per eye. Results are presented as mean $\pm$ SEM. Two-way ANOVA, Bonferroni post-hoc test, $*p < 0.05$. Scale bar, 20 μm .

Fig. 3. Circadian locomotor activity rhythms in SD rats versus P23H rats treated or untreated with HU210. Example of actograms (left panels), periodograms (middle panels), and mean waveforms (right panels) at P81-P90 for a SD rat (A), a control P23H rat (B) and a P23H rat

treated with HU210 (C), exposed all of them to a 12:12 LD cycle. Light and dark schedules are shown by white and black bars, respectively.

Fig. 4. Relationship between retinal and circadian parameters in P23H rats treated or untreated with cannabinoids. (A) Positive linear correlations between the maximum scotopic b-wave recorded on P90 P23H rats (treated or untreated with HU210) and the chronobiological parameter values obtained at the same age: amplitude (left), acrophase (middle) and interdaily phase stability (IS, right) of the circadian LA rhythms. (B) Correlations between the retinal ONL thickness in P90 P23H rats (treated or untreated with HU210) and circadian parameters of locomotor activity at the same age: amplitude (left), acrophase (middle) and interdaily phase stability (IS, right).

Table 1. Circadian parameters. Circadian parameters (mean $\pm$ SEM) of locomotor activity from SD and P23H rats, and Pearson's product moment correlation coefficients from the correlational analyses between circadian parameters and retinal measurements in P23H rats. Mesor, 24-h time series mean; amplitude, one-half the peak-to-trough variation of the 24-h rhythm; acrophase, peak time relative to local midnight; % variance, index of goodness of fit of the 24-h waveform approximation to the time series data; IS, interdaily stability; IV, intradaily variability; RA, relative amplitude; CFI, circadian function index. Student's *t* test, strain effect (vehicle-administered P23H vs. SD): $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$; Student's *t* test, treatment effect in P23H rats (HU210 vs. vehicle): $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$; Pearson's product moment correlation coefficients (circadian parameters vs. retinal measurements): $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$.

Table 1. Circadian parameters.

	SD N = 5	P23H N = 5	P23H+HU210 N = 6	Correlation with b-max	Correlation with ONL thickness
Rhythm parameters					
Mesor (counts)	8.01 ± 0.85	7.57 ± 0.27	9.86 ± 0.69*	0.58	0.65 [†]
Amplitude (counts)	7.81 ± 0.90	5.22 ± 0.19 [†]	8.93 ± 0.71**	0.66 [†]	0.63 [†]
Acrophase (min)	123.2 ± 26.8	50.1 ± 14.2	124.1 ± 8.2**	0.71 [†]	0.79 ^{††}
% Variance	39.3 ± 1.2	31.1 ± 1.8 ^{††}	38.8 ± 1.3*	0.47	0.54
Non-parametric variables					
IS	0.43 ± 0.05	0.27 ± 0.01 [†]	0.35 ± 0.01***	0.61 [†]	0.74 [†]
IV	1.11 ± 0.04	0.86 ± 0.03 ^{††}	1.00 ± 0.03*	0.71 [†]	0.69 [†]
RA	0.87 ± 0.04	0.83 ± 0.01 [†]	0.87 ± 0.01*	0.81 ^{††}	0.67 [†]
CFI	0.58 ± 0.21	0.56 ± 0.01	0.58 ± 0.00*	0.28	0.27

Circadian parameters (mean ± SEM) of locomotor activity from SD and P23H rats, and Pearson's product moment correlation coefficients from the correlational analyses between circadian parameters and retinal measurements in P23H rats. Mesor, 24-h time series mean; amplitude, one-half the peak-to-trough variation of the 24-h rhythm; acrophase, peak time relative to local midnight; % variance, index of goodness of fit of the 24-h waveform approximation to the time series data; IS, interdaily stability; IV, intradaily variability; RA, relative amplitude; CFI, circadian function index. Student's *t* test, strain effect (vehicle-administered P23H vs. SD): [†]*p* < 0.05, ^{††}*p* < 0.01; Student's *t* test, treatment effect in P23H rats (HU210 vs. vehicle): **p* < 0.05, ***p* < 0.01, ****p* < 0.001; Pearson's product moment correlation coefficients (circadian parameters vs. retinal measurements): [†]*p* < 0.05, ^{††}*p* < 0.01.

Highlights

Cannabinoid administration rescues retinal degeneration in dystrophic P23H rats

Locomotor activity circadian rhythmicity improves in cannabinoid-treated P23H rats

Cannabinoid-mediated retinal rescue correlates with improved circadian parameters