Understanding daily rhythms in weakly electric fish: the role of melatonin on the electric behavior of Brachyhypopomus gauderio

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Abstract

Living organisms display an array of molecular, physiological and behavioral rhythms synchronized with natural environmental cycles. Understanding these interactions gains power when the complexity of natural habitats and the diversity of behavioral and physiological adaptations are taken into account. *Brachyhypopomus gauderio*, a South American weakly electric fish, are characterized by the emission of electrical discharges (EOD), with a very stable rate that is modulated by social and environmental cues. The nocturnal arousal in *B. gauderio* coincides with a melatonin-dependent EOD rate increase. Here we first show a daily cycle in both the EOD-BR and EOD-BR variability of *B. gauderio* in nature. We approached the understanding of the role of melatonin on this natural behavior through both behavioral pharmacology and *in vitro* assays. We report, for the first time in gymnotiformes, a direct effect of melatonin on the PN *in vitro* preparation. Melatonin treatment lowered EOD-BR in freely moving fish and PN-BR, while increasing the variability of both. These results show that melatonin plays a key role in modulating the electric behavior of *B. gauderio* through its effect on rate and variability, both of which must be under a tight temporal regulation to prepare the animal for a challenging nocturnal environment.

Introduction

Living organisms display an array of molecular, physiological and behavioral rhythms, endogenous at core, which are synchronized with environmental cycles and social stimuli. Biological rhythms confer an evolutionary advantage, allowing individuals to change predictively, allocating energy more efficiently and preparing for cyclic changes in the environment. Circadian rhythms oscillate with a period close to 24 hours and are synchronized with environmental cycles (*zeitgebers*), mainly photoperiod, although other cycles such as thermoperiod can act as alternative zeitgebers (Kronfeld-Schor et al. 2013). Phase relationship between biological rhythms and environmental cycles need both stability and a certain degree of flexibility, which allows adjustments to small unpredictable variations. In vertebrates, information of environmental changes in light is conveyed to the brain as a rise in melatonin production and secretion that generates a concentration plateau that coincides with the duration of the night (Falcón et al. 2010), (Cipolla-Neto and Amaral 2018). Melatonin modulates biological rhythms in physiology and behavior. It is synthesized in the pineal organ, the main source of circulating hormone, although other secondary sites of production in the teleost brain have been described (Falcón et al. 2010). Teleosts express three distinct melatonin receptors: Mel1a and Mel1b, analogous to the mammalian MT1 and MT2 respectively, and Mel1c, which they share with amphibians, reptiles and birds (Reppert et al. 1995), all of which are G protein-coupled receptors (Dubocovich et al. 2010). These receptors are widely expressed throughout the brain, including mid-brain regions involved in the organization of social behavior, as well as in peripheral organs and tissues (Falcón et al. 2010; Isorna et al. 2017).

Daily activity patterns are perhaps the most conspicuous behavioral rhythms. Animals tend to occupy temporal niches and organize behavior accordingly. Understanding the interaction between environment, physiology and behavior gains power when approached taking into account the complexity of natural habitats and the diversity of behavioral and physiological adaptations. South American weakly electric

fish are nocturnal animals that emit electric discharges generated by a specialized electric organ. These electric organ discharges (EODs) are involved both in active electro-reception, perhaps the main sensory modality of these fish (von der Emde 2006), and intra-specific communication, where both waveform and frequency can encode information about an individual's species, sex, and reproductive status (Caputi et al. 2005; Zupanc and Bullock 2006). The EOD basal rate of emission (EOD-BR) is modulated by environmental and social information (Engler and Zupanc 2001; Stoddard et al. 2007; Silva et al. 2007) and is part of a behavioral display known as electric behavior (EB). The EOD-BR is directly controlled by the spontaneous discharge of a pacemaker nucleus of the brainstem (PN) (Bennett 1971) in a 1:1 relation, meaning that each EOD corresponds to a discharge of this nucleus. The PN receives modulatory central connections from pre-pacemaker nuclei as well as from other central structures (Kawasaki et al. 1988; Heiligenberg 1991; Quintana et al. 2014; Perrone et al. 2014; Borde et al. 2020). The PN is a stable oscillator with low-rate variability (Capurro et al. 1999a; Vitar 2019) and consequently the EOD-BR of isolated, resting fish is also very stable. *Brachyhypopomus gauderio* (Giora and Malabarba 2009), native to Uruguay, is a gregarious species where individuals form flexible social groups (Miranda et al. 2008). They forage, explore and interact with conspecifics during the night. This state of nocturnal arousal is accompanied by an increase in their EOD-BR synchronized with the dark-phase and modulated by social context in laboratory conditions (Stoddard et al. 2007; Silva et al. 2007; Jun et al. 2014). This nocturnal increase is known to be mediated by melatonin (Migliaro and Silva 2016), but it is unknown whether melatonin alone is sufficient to mimic the natural electric behavior or if this modulation is achieved through direct effect on the PN.

The dual role of EODs requires a robust yet flexible rhythm for animals to correctly sense the environment while leaving some leeway to allow for social and environmental modulations. A recent study from our group (Gascue, V. et al. 2020) has shown that in the sympatric species *Gymnotus omarorum*, both day-time and social context influence EOD-BR variability in a consistent manner. Therefore, EOD-BR variability provides a great metric into the ecological and social pressures the fish are under, although it has not traditionally been studied as a behavioral trait in itself.

Using electrophysiology and behavioral recordings (both in the field and in the lab) in this study we confirm: i) the daily rhythmicity of two distinct traits of the electric behavior of *B. gauderio* (EOD-BR and EOD-BR variability) in the wild, and ii) the melatoninergic modulation of electric behavior through its action on the central command.

Materials And Methods

Animals

Adult *B. gauderio* were either registered in the natural habitat (n = 6), used in behavioral experiments (n = 23) or in *in vitro* recordings (n = 6). All specimens were collected in Laguna Lavalle, Depto. de Tacuarembó (32°01'21.8"S 55°22'35.6"W). Fish were identified using an electronic audio amplifier

connected to a pair of electrodes as described elsewhere (Silva et al. 2003). Animals used in laboratory experiments were housed in outdoor tanks and fed with *Tubifex tubifex*.

Behavioral recordings in nature.

Animals in the natural habitat were recorded during the non-breeding season at the peri-equinox period, under a natural light-dark cycle of 12:12. Periodic light and temperature measures were taken every 30 min, inside the water under the natural vegetation and outside the water (HOBO-MicroDAQ: UA-002-08 Measurement range: temperature, − 20° to 70°C; light, 0 to 320,000 lux or 0 to 30,000 lumens/ft2). EOD-BR was recorded from fish placed in plastic mesh enclosures, which allow water interchange, equipped with electrodes. Nets were kept under the natural vegetation for 72 h. EOD-BR was recorded for 30s every hour. Fish in this condition are almost always detectable, able to move around and exposed to neighboring fish electric signals, although long range swimming was restricted.

In order to normalize the effect of water temperature on EOD-BR, values were corrected to a constant 20°C temperature by using the Q10 value of 1.5 as calculated for electric fish (Dunlap et al. 2000; Silva et al. 2007).

Q10 = BR x (T)/ BR x (T + 10)

Pharmacological preparations

Melatonin stock dilution

5mg melatonin (SIGMA, 98%) were dissolved in 100µl ethanol and diluted in either in 400µl of saline solution (behavioral experiments, 0.9% NaCl) or fish Ringer solution (*in vitro* experiments, following (Perrone et al. 2010)). For control experiments purposes a vehicle solution was made using 400µl of saline/ringer solution and 100µl of ethanol.

Ringer-sucrose solution: in mM: 213 sucrose, 3 KCl, 0.75 KH_2PO_4, 1.2 $MgSO_4$, 24 $NaHCO_3$,10 D-glucosa, 1.6 $CaCl_2$, pH 7.2–7.4 after carbogen saturation.

Ringer solution

in mM 124 NaCl, 3 KCl, 0.75 KH_2PO_4, 1.2 $MgSO_4$, 1.6 $CaCl_2$,24 NaH_2CO_3, and 10 D-glucosa, pH = 7.4 after carbogen saturation.

Behavioral experiments

Fish were placed in individual 40l (55cm × 40cm × 25cm) tanks inside a recording chamber that allowed electric recordings as described elsewhere (Silva et al. 2007). The chamber was kept in a 12L:12D light cycle, and temperature (20°C) and water conductivity (≈ 100µS) were kept constant according to natural conditions (Silva et al. 2003). We recorded EODs for 1h to obtain base-line values, and then injected the

fish intraperitoneally (IP) with: a) 100µL of control solution (n = 9) or a melatonin solution of either b) 5µg/gbw (n = 7) or c) 50µg/gbw (n = 7) (Pinillos et al. 2001). EODs were recorded for two more hours.

Recordings were analyzed using Clampfit (v.10.2, Molecular Devices). Recordings were sampled for 1 minute every 10 minutes, and the timing of EOD events was obtained using a threshold protocol. The mean frequency, standard deviation (SD), and coefficient of variation (CV) were obtained for every sample taken, and the EOD-BR rates (mean ± SD) were plotted against time. In order to compare the effects obtained on either different individuals or different moments, EOD-BR values of a given time (t) were normalized against the values calculated 10 minutes before the injection (t=-10), using the following equation:

EODBRin(t) = EODBR(t) / EODBR(-10)

In vitro experiments

Fish (n = 6) were anesthetized between 10:00 and 12:00hs as described elsewhere (Perrone et al. 2010) and brain-stem slices (600–700 µm thick) containing the PN were immediately obtained under iced ringer-sucrose solution. Each slice was maintained in a 4ml electro-physiological recording chamber superfused with oxygenated Ringer solution at room temperature. Under these conditions the PN maintains spontaneous activity for several hours (Quintana et al. 2014). PN spontaneous activity was assessed by recording the field potential generated by the activation of a population of pacemaker and relay cells close to the micropipette. Recordings were made using a low resistance glass micropipette (3–5 MΩ) close to the PN, filled with Ringer solution. Signals were amplified (AM Systems, M3000, filters 150–3000 Hz) and digitized (Digidata 1440, Molecular Devices) for further analysis. A 1-hour period of basal activity was recorded (1 minute every 10 minutes) prior to the following pharmacological treatment: either 500µl of a 1.25mg/ml melatonin solution or 500µl of a control solution consisting of the melatonin vehicle was added to an anterior chamber and driven to the recording chamber by the flow of Ringer solution.

Recordings were analyzed with Clampfit. The timing of PN spike events was obtained using a threshold protocol to obtain instantaneous frequency values. Mean frequency (PN-BR), SD and CV were obtained for each sample taken. In order to compare between experiments a basal frequency index (PN-BRin) was obtained for each brain slice using the following equation:

PNBRin(t) = PNBR(t) / PNBR(-10)

Variability analysis

Variability of EOD-BR (EOD-BRvar) and PN-BR (PN-BRvar) was analyzed using two complementary approaches: a traditional variability parameter, the coefficient of variation (CV) and a Poincaré plot's analysis for a qualitative and quantitative description of our data. Both sets of analyses were performed by an ad-hoc Matlab (The MathWorks, Inc) routine.

Coefficient of variation (CV) was calculated as follows (where δ is the calculated standard deviation, and X is the mean, for EOD-BR o PN-BR values):

$CV = \delta / X$

Poincaré diagrams were plotted as a means to obtain a qualitative analysis of dispersion. Each observed value of a set of data is plotted against the following, i.e., each EOD's instant frequency value (EOD-BR) against the following (EOD-BR + 1). This type of diagrams is intimately related to the system's variability which can be quantified by an ellipse fitted to the graph using least square criterion. The perpendicular (SD1) and longitudinal (SD2) axes of this ellipse represent short- and long-term variability (cycle to cycle and global variability) of the system respectively (Fishman et al. 2012).

Statistical analysis

The analysis of the daily rhythm in the nocturnal increase in EOD-BR was carried out using a cosinor fit (Campuzano et al. 1999). Cosinor gives a statistical validation for the fitting of a cosine function within a 24 h period to each fish EOD rate data, as well as the acrophase value in lineal hours for each fish (transformed to clock hours for simplicity). Population acrophase was calculated and statistically validated using the Rayleigh test with the individual acrophases calculated as described (Refinetti et al. 2007).

Non-parametric tests (Kruskal-Wallis test followed by Dunn's post hoc protocol, Mann-Whitney U test, Wilcoxon test) were used in all following analyses and are specified when data is shown. When comparing different treatment groups at different times, a mixed effects model analysis was used followed by Dunn's post hoc analysis to check for differences between groups. All research procedures complied with ASAP/ABS Guidelines for the Use of Animals in Research and were approved by the Institutional Ethical Committee (Comisión de Ética en el Uso de Animales: Instituto Clemente Estable: 008/11; Facultad de Ciencias: 1304, 1325).

Results

Daily modulations of EOD-BR in nature

Daily changes in EOD-BR recorded in nature reflect the outcome of the circadian system interacting with a cycling environment. Assessing the daily changes of electric behavior in the wild is mandatory for understanding the natural outcome of a nocturnal physiology that emerges in synchrony with dusk. Water temperature cycled as expected, rising through the day and reaching the maximum value at dusk (as shown in (Migliaro et al. 2018)). Figure 1 shows individual (n = 6) EOD-BR changes over 72 hs. Electric behavior has a clear daily rhythm, rising towards dusk (7 PM) and decaying towards dawn (7 AM) (Fig. 1A). Mean population EOD-BR at dusk was 46% higher than 60 minutes before (Dusk − 60). By dawn, EOD-BR values were 144% lower than at dusk, and 67% lower than at Dusk-60. (Fig. 1B; Kruskal-Wallis test, n = 6, $p < 0.0001$; Dunn's post hoc: EOD-BR at dusk vs EOD-BR 60 min. before dusk; n = 6, $p = 0.05$;

EOD-BR at dusk vs EOD-BR at dawn, n = 6, p = 0.0001; EOD-BR 60 min. before dusk vs EOD-BR at dawn, n = 6, p = 0.05). Cosinor analysis confirmed the daily rhythmicity of the nocturnal increase in EOD-BR, with individual acrophases synchronized to the natural dusk and the moment of maximum water temperature (Fig. 1C, n = 6, Rayleigh test, p = 0,0003).

Interestingly the nocturnal increase in EOD-BR was paired with a nocturnal increase in its variability (EOD-BR_{var}). Figure 2 shows a 60% increase in CV at night compared to daytime values. (Fig. 2A, n = 6, Wilcoxon test, p = 0,031). Figure 2B shows an individual Poincaré diagram for nocturnal and diurnal instant EOD-BR. Statistical analysis of the parameters quantified for each individual shows an 35% increase in nocturnal short-term variability (SD1day vs. SD1night, n = 6, Wilcoxon test, p = 0,046). No statistical differences were found for long term variability (SD2d vs. SD2n, n = 6, Wilcoxon test, p = 0,81).

Melatonin actions on electric behavior

Acute intraperitoneal treatment with melatonin during daytime consistently lowered EOD-BR in a dose-dependent manner. Figure 3A shows the temporal dynamics of EOD-BR change relative to EOD-BR 10 minutes before treatment (measured by the EOD-BRin), for control and melatonin treated (50µg/gbw dose) animals. Time to maximum effect, evidenced by minimum EOD-BR value, varied among individuals ranging from 15 to 70 minutes after injection. However, a statistical analysis showed significant differences in comparison to control animals, from 20 to 120 minutes after injection (Mixed model effects analysis with Dunn's post hoc for multiple comparisons. Both treatment group and the interaction between time and treatment group had a significant effect, $p < 0.0001$). Figure 3B shows EOD-BRin for the experimental and control groups in three different time windows: one hour before treatment, first hour post treatment and second hour post treatment. Melatonin-treated animals showed a 30% decrease in the mean EOD-BRin during the first hour post injection compared to control animals (Mann-Whitney U test, control n = 9 vs Mel. n = 7, p = 0,04). This effect tended to wear out during the second hour, although it still maintains a 25% of decrease (Mann-Whitney U test, control n = 9 vs Mel. n = 7, p = 0,017). No differences were found during the pre-treatment hour (Mann-Whitney U test, control n = 9 vs Mel. n = 7, p = 0,66) (Fig. 3B). In order to analyze the maximum effect of melatonin treatment, EOD-BR indexes were calculated at the time of maximum effect (minimum EOD-BR) for each fish. For control experiments we used the moment of minimum EOD-BR value in the 120 minute post-injection period. At the moment of maximum effect (time of minimum EOD-BR for each animal) EOD-BRin for the treated group showed a 34% decrease (Dunn's post hoc, Control n = 9 vs Mel. 50µg/gbw n = 7, $p < 0.0001$). A second experimental group was treated with a lower melatonin dose (5µg/gbw). The significant effect of the lower dose at the moment of maximum effect was similar in magnitude to the higher one, as depicted in Fig. 3C (Dunn's post hoc, Control n = 9 vs Mel. 5µg/gbw n = 7, p = 0.0240). This effect however had a shorter duration, as EOD-BR returned to basal values before one hour (data not shown).

Melatonin effect on the variability of EOD-BR

Melatonin effect on electric behavior goes beyond the modulation of EOD-BR and includes a clear effect on EOD-BR variability (EOD-BRvar). While melatonin decreased EOD-BR it increased EOD-BRvar. Figure 4 shows the melatonin effect on global and cycle-to-cycle variability (CV and SD1 respectively). Data is shown as the ratio between post (120 minutes) and pre-treatment (60 minutes) variability values for both experimental conditions (control/melatonin). Melatonin treatment (50 µg/gbw) caused an increase in the CV ratio of 440% and an increase in SD1post/SD1pre values of 120%, while control animals showed no change in any of their variability measurements (Mann-Whitney test, control n = 9, Mel. n = 7, p = 0.001 and p = 0.016 respectively). SD2 values also increased 100% times in treated animals (Mann-Whitney test, Control n = 9, Mel. n = 7, p = 0.031). Animals treated with the 5µg/gbw melatonin dose showed no changes in their EOD-BRvar.

Melatonin effect in the central command for electric behavior

Melatonin modulates the PN´s spontaneous activity. Melatonin perfusion induces a decrease in PN-BR. An individual example is shown in Fig. 5A, including a control stage (white arrow marks the administration of vehicle solution to the anterior chamber) followed by a treatment stage (red arrow marks the addition of melatonin solution to the anterior chamber). Discharge rate decreased progressively reaching significantly lower values 40 to 60 minutes after starting melatonin perfusion, reaching a rate 63% lower than its original values (Fig. 5B, Dunn's post hoc, n = 6, p = 0.027 and p = 0.005 at 40 and 60 minutes respectively). Melatonin effect was reverted by continuous perfusion with the standard ringer solution, and PN-BR values were non-distinct from basal values 20 minutes after washing (Dunn’s post hoc, n = 6, p = 0.407). PN-BRin in melatonin-perfused slices was significantly decreased 60 minutes after perfusion while no effect is shown in control experiments. (Fig. 5C, Mann-Whitney test, Control n = 4, Mel. n = 6, p = 0.014).

Melatonin effect on the variability of pacemaker nucleus spontaneous discharge

Similarly to the effect on electric behavior, there was a tendency of melatonin treatment to increase PN-BRvar. (Fig. 6). Melatonin increases on PN-BRvar were marginal, most probably due a small experimental number (Wilcoxon’s, n = 4, p = 0.068). Only four out of the six melatonin treatment recordings were stable enough before perfusion to allow a robust analysis of the spontaneous rate variability. Experimental manipulation generated a slight increase in variability, which is evident in two of the control experiments, however the variability of the spontaneous discharge was consistently increased by melatonin treatment.

Discussion

Nocturnality is a behavioral trait characterized by the allocation of increased awareness and general activity (foraging, social interactions, territory patrolling) in a time window synchronized with the dark

phase of the photoperiod, the night. This synchronization reflects entrainment of endogenous circadian rhythms to environmental daily cycles and mediates the colonization of a specific temporal niche. Freshwater South American weakly electric fish are nocturnal animals. Nocturnality has been explored in several species of this group, with behavioral approaches that focus on locomotor activity, electric behavior and electrocommunication. Early experiments showed a nocturnal increase in locomotor activity and exploratory behavior (Lissmann and Schwassmann 1965; Black-Cleworth 1970). More recent reports demonstrated the circadian nature of nocturnal variations in electric behavior, EOD-Br increases and social electric signals are more frequent during the night or subjective night (Engler and Zupanc 2001; Stoddard et al. 2007), provided social context is maintained. Due to EOD's joint role as the carrier of sensory stimuli and communicative signals, changes in EOD rate show different states of awareness, novelty detection, social engagement and attention across different Gymnotiform species (Caputi et al. 2003; Silva et al. 2007; Jun et al. 2014, 2016; Perrone and Silva 2018; Gascue, V. et al. 2020). Behavioral recordings in *G. omarorum* have shown that the increase in EOD-Br is a natural trait, independent of exploratory behavior and persistent under natural constant darkness conditions (Migliaro et al. 2018).

Our results show that the electric behavior of *B. gauderio*, recorded in the natural habitat, has a daily rhythm of EOD-Br characterized by a steep increase in the afternoons, with a peak close to sunset, and a slow decay during the night towards sunrise. Individual acrophases are synchronized with the moment of maximal water temperature values, similarly to the behavior previously described for *G. omarorum* (Migliaro et al. 2018). Given the nocturnal habits of these fish, EOD-Br is expected to increase at night, as we observe in our results. Our data also shows another behavioral trait associated with the night, the increase in EOD-Br variability in both global and cycle to cycle measurements. This increase in rate variability reflects a wider range of inter-EOD interval duration (and hence of instantaneous frequency values), which is to be expected if animals are interacting and exploring their environment. It is interesting to consider whether this nocturnal increase in variability reflects an endogenous mechanism or is a consequence of the nocturnal increase in activity and awareness. EOD-Br variability has been reported to be very low in isolated resting gymnotiforms in laboratory conditions (Capurro et al. 1999b; Vitar 2019), contrasting with the natural daily modulation reported for *G. omarorum* (Gascue, V. et al. 2020) and for *B. gauderio* in the present work.

Information about the beginning and duration of the night is relayed to the vertebrate brain by the melatoninergic system. The circadian rhythm of endogenous melatonin production is synchronized with the night due to the inhibitory effect of the environmental light. In the present report we demonstrate that melatonin modulates electric behavior in both EOD-Br and EOD-BR variability. Moreover, electric behavior modulation is a consequence of the melatonin action on the discharge rate of the pacemaker nucleus and its variability. The similarity between the melatoninergic modulation of electric behavior and pacemaker activity suggests the central origin of this modulation. Melatonin decreases PM discharge rate and consequently EOD-Br, while increasing PN discharge rate variability with the concurrent increase in EOD-Br variability, showing an independent regulation of each trait.

Melatonin modulation of EOD-BR.

Our data shows that melatonin consistently decreases EOD-Br and that this influence is exerted at the level of the PN, through direct action on the *in vitro* preparation. This decrease in discharge rate seems at odds with the reported nocturnal increase in EOD-Br in both laboratory and natural conditions (Silva et al. 2007; Migliaro and Silva 2016). Moreover, melatonin is a key determinant of the nocturnal increase itself (Migliaro and Silva 2016). Melatonin actions on behavior and physiology are widely variable and context dependent. At the neuronal level melatonin has been reported to decrease excitability in different areas of the central nervous system as the cerebellum, suprachiasmatic nucleus or dorsal root ganglions through regulation of a delayed rectifier potassium current, modulation of GABAergic transmission, attenuation of the sodium current and also an interaction with passive membrane properties (Huan et al. 2001; Scott et al. 2010; Oliveira-Abreu et al. 2018). In this sense a decrease in the frequency of pacemaker activity is to be expected, as has been reported for other nuclei with oscillatory properties (Jiang et al., 1995; Mason and Brooks, 1988; Oliveira-Abreu et al., 2019; Scott et al., 2010; Shibata et al., 1989; Stehle et al., 1989). Moreover, this modulation has a time-of-the-day dependent outcome, due to circadian changes in the expression of melatonin receptors (Gaildrat et al. 1998; Ikegami et al. 2009; Feng et al. 2015). Mel1b receptor expression has been shown to be widespread in brain areas involved in vocal signaling in the midshipman fish (Feng and Bass 2016; Feng et al. 2019), a communication system which shares cellular, circuital and functional characteristics with the electrogenic system in fish. In this system melatonin receptors are expressed in bulbar nuclei as well as in upstream midbrain nuclei. This shows that melatonin regulation is a complex, multicomponent system with multiple pathways.

Electric behavior is the result of a number of influences converging in the PN that are in turn modulated by different factors with different temporal dynamics, among them are melatonin concentration and melatonin receptor expression. The buildup in the nocturnal melatonin concentration has species specific profiles across vertebrates (Falcón et al. 2010). In humans the physiological parameter indicating the timing of melatonin increase is called DLMO (dim light melatonin onset), and precedes the expression of nocturnal behavior in this diurnal species. The DLMO concept can be extended to other vertebrate species given the highly conserved features of the melatoninergic system. This holds true, even when environmental light is not a robust cyclic cue as happens with animals living in constant darkness or humans with retinohypothalamic tract damage (Cipolla-Neto and Amaral 2018). In this way the melatonin onset (MO) precedes the acrophase of a rhythmic behavior that is being modulated by melatonin itself. Hence, it is interesting to take a closer look at the natural nocturnal increase in EOD-BR, which has an acrophase at sunset that gradually declines towards sunrise. The melatonin onset might be working as a timing signal, affecting nuclei of the midbrain that are upstream from the PN, inducing the increase in EOD-BR. This increase will reach a maximum when the direct effect of melatonin on the PN starts counterbalancing the initial increase, generating the progressive decrease towards sunrise. This dual role as hormonal time giver and modulator of cellular excitability needs further confirmation, especially regarding melatonin onset in natural conditions.

Melatonin modulation of EOD-Br variability.

Gymnotiforms when isolated, unperturbed and resting are incredibly stable oscillators with respect to their electric behavior as well as to the spontaneous activity of the PN (Moortgat et al. 2000; Vitar 2019). This makes functional sense, as these animals require a robust system capable of withstanding external influences so as to not jeopardize their sensory capabilities. This stability however might be in conflict with the communication and cognitive capabilities of these animals, as using their EODs as a communication channel requires these animals to modulate their frequency in dynamic ways, usually within milliseconds after receiving a signal from a conspecific (Perrone et al. 2009). Exploring and navigating the world also demands rapid adjustments in EOD rate (Caputi et al. 2003; Jun et al. 2014).

In our behavioral experiments, melatonin produced an almost 10 time increase in the CV of EOD-Br. We can then assert melatonin's role in the modulation of variability of electric behavior, mimicking the nocturnal increase in EOD-Br variability in the natural habitat. This increase in EOD-Br variability results from the increase in variability produced by melatonin in the central pacemaker, as shown in our results in comparison to saline treated preparations. An increase in variability allows for more flexible, rapid changes in the PN rate and hence in EOD-Br, as the ones that sustain social electric signals (Quintana et al. 2011, 2014; Comas et al. 2019).

This is the first report of the influence of melatonin on behavior in electric fish. In the present study we show a melatoninergic modulation of two traits of electric behavior, both of which must be under tight temporal regulation to allow for the correct behavioral display. This temporal association of processes through the action of the same modulator is common in systems where the temporal synchronization of multiple independent parts is crucial (Adkins-Regan 2013).

Declarations

The authors declare no competing or financial interests

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References

1. Adkins-Regan E (2013) Hormones and animal social behavior. In: Hormones and Animal Social Behavior. Princeton University Press
2. Bennett MVL (1971) Electric organs. In: Fish physiology. Elsevier, pp 347–491
3. Black-Cleworth P (1970) The role of electrical discharges in the non-reproductive social behaviour of Gymnotus carapo (Gymnotidae, Pisces). Animal Behaviour Monographs 3:1-IN1

4. Borde M, Quintana L, Comas V, Silva A (2020) Hormone-mediated modulation of the electromotor CPG in pulse-type weakly electric fish. Commonalities and differences across species. Developmental Neurobiology 80:70–80
5. Campuzano A, Cambras T, Vilaplana J, et al (1999) Period Length of the Light–Dark Cycle Influences the Growth Rate and Food Intake in Mice. Physiology & Behavior 67:791–797. https://doi.org/10.1016/S0031-9384(99)00196-1
6. Capurro A, Pakdaman K, Perrone R, Macadar O (1999a) Analysis of the jamming avoidance response in the electric fish Gymnotus carapo. Biological cybernetics 80:269–283
7. Capurro A, Pakdaman K, Perrone R, Macadar O (1999b) Analysis of the jamming avoidance response in the electric fish Gymnotus carapo. Biological cybernetics 80:269–283
8. Caputi AA, Aguilera PA, Castelló ME (2003) Probability and amplitude of novelty responses as a function of the change in contrast of the reafferent image in G. carapo. Journal of Experimental Biology 206:999–1010
9. Caputi AA, Carlson BA, Macadar O (2005) Electric organs and their control. In: Electroreception. Springer, pp 410–451
10. Cipolla-Neto J, Amaral FG do (2018) Melatonin as a Hormone: New Physiological and Clinical Insights. Endocrine Reviews 39:990–1028. https://doi.org/10.1210/er.2018-00084
11. Comas V, Langevin K, Silva A, Borde M (2019) Distinctive mechanisms underlie the emission of social electric signals of submission in Gymnotus omarorum. Journal of Experimental Biology 222: jeb195354. https://doi.org/10.1242/jeb.195354
12. Dubocovich ML, Delagrange P, Krause DN, et al (2010) International Union of Basic and Clinical Pharmacology. LXXV. Nomenclature, classification, and pharmacology of G protein-coupled melatonin receptors. Pharmacological reviews 62:343–380
13. Dunlap KD, Smith GT, Yekta A (2000) Temperature Dependence of Electrocommunication Signals and Their Underlying Neural Rhythms in the Weakly Electric Fish, Apteronotus leptorhynchus. BBE 55:152–162. https://doi.org/10.1159/000006649
14. Engler G, Zupanc G (2001) Differential production of chirping behavior evoked by electrical stimulation of the weakly electric fish, Apteronotus leptorhynchus. Journal of Comparative Physiology A 187:747–756
15. Falcón J, Migaud H, Muñoz-Cueto JA, Carrillo M (2010) Current knowledge on the melatonin system in teleost fish. General and Comparative Endocrinology 165:469–482. https://doi.org/10.1016/j.ygcen.2009.04.026
16. Feng NY, Bass AH (2016) “Singing” fish rely on circadian rhythm and melatonin for the timing of nocturnal courtship vocalization. Current Biology 26:2681–2689
17. Feng NY, Fergus DJ, Bass AH (2015) Neural transcriptome reveals molecular mechanisms for temporal control of vocalization across multiple timescales. BMC genomics 16:1–25
18. Feng NY, Marchaterre MA, Bass AH (2019) Melatonin receptor expression in vocal, auditory, and neuroendocrine centers of a highly vocal fish, the plainfin midshipman (Porichthys notatus). Journal

of Comparative Neurology 527:1362–1377

19. Fishman M, Jacono FJ, Park S, et al (2012) A method for analyzing temporal patterns of variability of a time series from Poincare plots. Journal of Applied Physiology 113:297–306
20. Gaildrat P, Ron B, Falcón J (1998) Daily and Circadian Variations in 2-[125I]-Iodomelatonin Binding Sites in the Pike Brain (Esox lucius). Journal of Neuroendocrinology 10:511–517. https://doi.org/10.1046/j.1365-2826.1998.00240.x
21. Gascue, V., Silva, Ana, Migliaro, Adriana (2020) Social modulation on daily variability in electric behavior. Sleep Science. https://doi.org/10.5935/1984-0063.20200012
22. Giora J, Malabarba LR (2009) Brachyhypopomus gauderio Giora & Malabarba, 2009, new species. https://doi.org/10.5281/zenodo.6213867
23. Heiligenberg W (1991) Sensory control of behavior in electric fish. Current opinion in neurobiology 1:633–637
24. Huan C, Zhou M, Wu M, et al (2001) Activation of melatonin receptor increases a delayed rectifier K+ current in rat cerebellar granule cells. Brain research 917:182–190
25. Ikegami T, Motohashi E, Doi H, et al (2009) Synchronized diurnal and circadian expressions of four subtypes of melatonin receptor genes in the diencephalon of a puffer fish with lunar-related spawning cycles. Neuroscience Letters 462:58–63. https://doi.org/10.1016/j.neulet.2009.06.076
26. Isorna E, de Pedro N, Valenciano AI, et al (2017) Interplay between the endocrine and circadian systems in fishes. Journal of Endocrinology 232:R141–R159
27. Jiang Z-G, Nelson CS, Allen CN (1995) Melatonin activates an outward current and inhibits Ih in rat suprachiasmatic nucleus neurons. Brain research 687:125–132
28. Jun JJ, Longtin A, Maler L (2014) Enhanced sensory sampling precedes self-initiated locomotion in an electric fish. J Exp Biol 217:3615–3628. https://doi.org/10.1242/jeb.105502
29. Jun JJ, Longtin A, Maler L (2016) Active sensing associated with spatial learning reveals memory-based attention in an electric fish. Journal of Neurophysiology 115:2577–2592. https://doi.org/10.1152/jn.00979.2015
30. Kawasaki M, Maler L, Rose GJ, Heiligenberg W (1988) Anatomical and functional organization of the prepacemaker nucleus in gymnotiform electric fish: the accommodation of two behaviors in one nucleus. Journal of Comparative Neurology 276:113–131
31. Kronfeld-Schor N, Bloch G, Schwartz WJ (2013) Animal clocks: when science meets nature. Proceedings of the Royal Society B: Biological Sciences 280:20131354
32. Lissmann HW, Schwassmann HO (1965) Activity rhythm of an electric fish, Gymnorhamphichthys hypostomus, Ellis. Zeitschrift für vergleichende Physiologie 51:153–171
33. Mason R, Brooks A (1988) The electrophysiological effects of melatonin and a putative melatonin antagonist (N-acetyltryptamine) on rat suprachiasmatic neurones in vitro. Neuroscience letters 95:296–301

34. Migliaro A, Moreno V, Marchal P, Silva A (2018) Daily changes in the electric behavior of weakly electric fish naturally persist in constant darkness and are socially synchronized. Biol Open 7:bio036319. https://doi.org/10.1242/bio.036319
35. Migliaro A, Silva A (2016) Melatonin Regulates Daily Variations in Electric Behavior Arousal in Two Species of Weakly Electric Fish with Different Social Structures. Brain Behav Evol 87:232–241. https://doi.org/10.1159/000445494
36. Miranda M, Silva AC, Stoddard PK (2008) Use of space as an indicator of social behavior and breeding systems in the gymnotiform electric fish Brachyhypopomus pinnicaudatus. Environmental Biology of Fishes 83:379–389. https://doi.org/10.1007/s10641-008-9358-2
37. Moortgat KT, Bullock TH, Sejnowski TJ (2000) Precision of the pacemaker nucleus in a weakly electric fish: network versus cellular influences. Journal of neurophysiology 83:971–983
38. Oliveira-Abreu K, Ferreira-da-Silva FW, da Silva-Alves KS, et al (2018) Melatonin decreases neuronal excitability in a sub-population of dorsal root ganglion neurons. Brain research 1692:1–8
39. Oliveira-Abreu K, Silva-dos-Santos NM, Coelho-de-Souza AN, et al (2019) Melatonin Reduces Excitability in Dorsal Root Ganglia Neurons with Inflection on the Repolarization Phase of the Action Potential. International Journal of Molecular Sciences 20:2611. https://doi.org/10.3390/ijms20112611
40. Perrone R, Batista G, Lorenzo D, et al (2010) Vasotocin Actions on Electric Behavior: Interspecific, Seasonal, and Social Context-Dependent Differences. Frontiers in Behavioral Neuroscience 4:
41. Perrone R, Macadar O, Silva A (2009) Social electric signals in freely moving dyads of Brachyhypopomus pinnicaudatus. Journal of Comparative Physiology A 195:501–514
42. Perrone R, Migliaro A, Comas V, et al (2014) Local vasotocin modulation of the pacemaker nucleus resembles distinct electric behaviors in two species of weakly electric fish. Journal of Physiology-Paris 108:203–212. https://doi.org/10.1016/j.jphysparis.2014.07.007
43. Perrone R, Silva AC (2018) Status-dependent vasotocin modulation of dominance and subordination in the weakly electric fish Gymnotus omarorum. Frontiers in Behavioral Neuroscience 12:1
44. Pinillos ML, De Pedro N, Alonso-Gómez AL, et al (2001) Food intake inhibition by melatonin in goldfish (Carassius auratus). Physiology & Behavior 72:629–634. https://doi.org/10.1016/S0031-9384(00)00399-1
45. Quintana L, Harvey-Girard E, Lescano C, et al (2014) Sex-specific role of a glutamate receptor subtype in a pacemaker nucleus controlling electric behavior. Journal of Physiology-Paris 108:155–166
46. Quintana L, Pouso P, Fabbiani G, Macadar O (2011) A central pacemaker that underlies the production of seasonal and sexually dimorphic social signals: anatomical and electrophysiological aspects. Journal of Comparative Physiology A 197:75–88
47. Refinetti R, Lissen GC, Halberg F (2007) Procedures for numerical analysis of circadian rhythms. Biol Rhythm Res 38:275–325. https://doi.org/10.1080/09291010600903692
48. Reppert SM, Weaver DR, Cassone VM, et al (1995) Melatonin receptors are for the birds: Molecular analysis of two receptor subtypes differentially expressed in chick brain. Neuron 15:1003–1015.

https://doi.org/10.1016/0896-6273(95)90090-X

49. Scott FF, Belle MDC, Delagrange P, Piggins HD (2010) Electrophysiological effects of melatonin on mouse Per1 and non-Per1 suprachiasmatic nuclei neurones in vitro. Journal of neuroendocrinology 22:1148–1156
50. Shibata S, Cassone VM, Moore RY (1989) Effects of melatonin on neuronal activity in the rat suprachiasmatic nucleus in vitro. Neuroscience letters 97:140–144
51. Silva A, Perrone R, Macadar O (2007) Environmental, seasonal, and social modulations of basal activity in a weakly electric fish. Physiol Behav 90:525–536. https://doi.org/10.1016/j.physbeh.2006.11.003
52. Silva A, Quintana L, Galeano M, Errandonea P (2003) Biogeography and Breeding in Gymnotiformes from Uruguay. Environmental Biology of Fishes 66:329–338. https://doi.org/10.1023/A:1023986600069
53. Stehle J, Vanecek J, Vollrath L (1989) Effects of melatonin on spontaneous electrical activity of neurons in rat suprachiasmatic nuclei: an in vitro iontophoretic study. Journal of Neural Transmission/General Section JNT 78:173–177
54. Stoddard PK, Markham MR, Salazar VL, Allee S (2007) Circadian rhythms in electric waveform structure and rate in the electric fish Brachyhypopomus pinnicaudatus. Physiol Behav 90:11–20. https://doi.org/10.1016/j.physbeh.2006.08.013
55. Vitar M (2019) El núcleo marcapaso de gymnotus omarorum: caracterización de la variabilidad de un modelo de oscilador neural en vertebrados
56. von der Emde G (2006) Non-visual environmental imaging and object detection through active electrolocation in weakly electric fish. Journal of Comparative Physiology A 192:601–612
57. Zupanc GKH, Bullock TH (2006) Walter Heiligenberg: the jamming avoidance response and beyond. J Comp Physiol A 192:561–572. https://doi.org/10.1007/s00359-006-0098-5
58. Melatonin Modulates Spike Coding in the Rat Suprachiasmatic Nucleus - Inyushkin - 2007 - Journal of Neuroendocrinology - Wiley Online Library. https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1365-2826.2007.01574.x. Accessed 23 Nov 2022

Figures

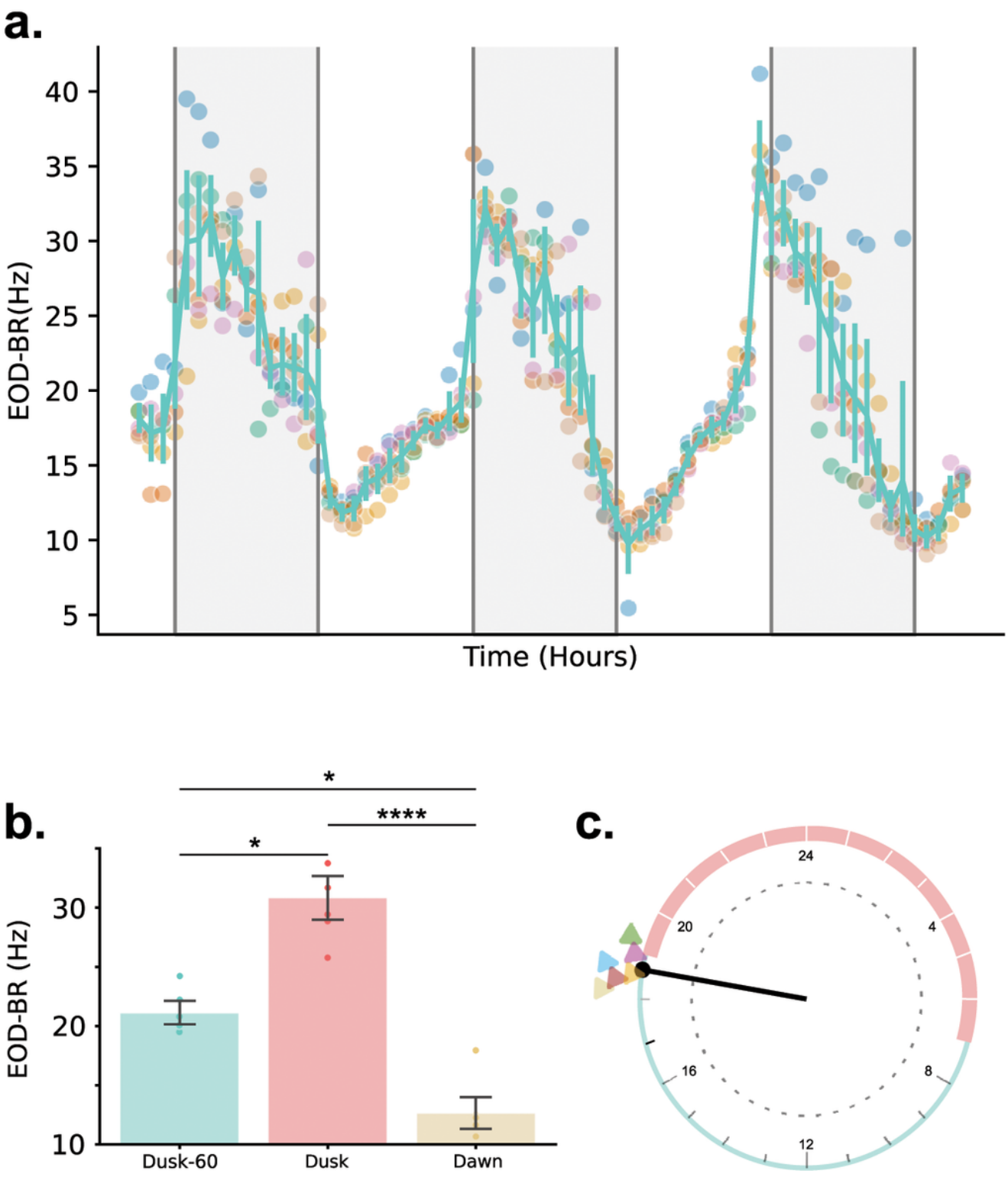

Figure 1

Daily changes in EOD-BR in a population in the natural habitat **a**. Mean hourly EOD-BR over 72 hours (n=6). Dots: mean EOD-BR for each fish; blue line: mean population EOD-BR; error bars: standard deviation; nights highlighted in gray. **b**. Mean population EOD-BR at dusk (7pm), an hour before dusk (Dusk-60) and at dawn (7am). **c**. Rayleigh test for EOD-BR showing a significant daily rhythmicity. Night

hours are highlighted in red. Each arrowhead signals the acrophase for an individual. The black radial line shows the interval of confidence for the test. *=p≤0.05, **=p≤0.01, ***=p≤0.001, ****=p≤0.0001

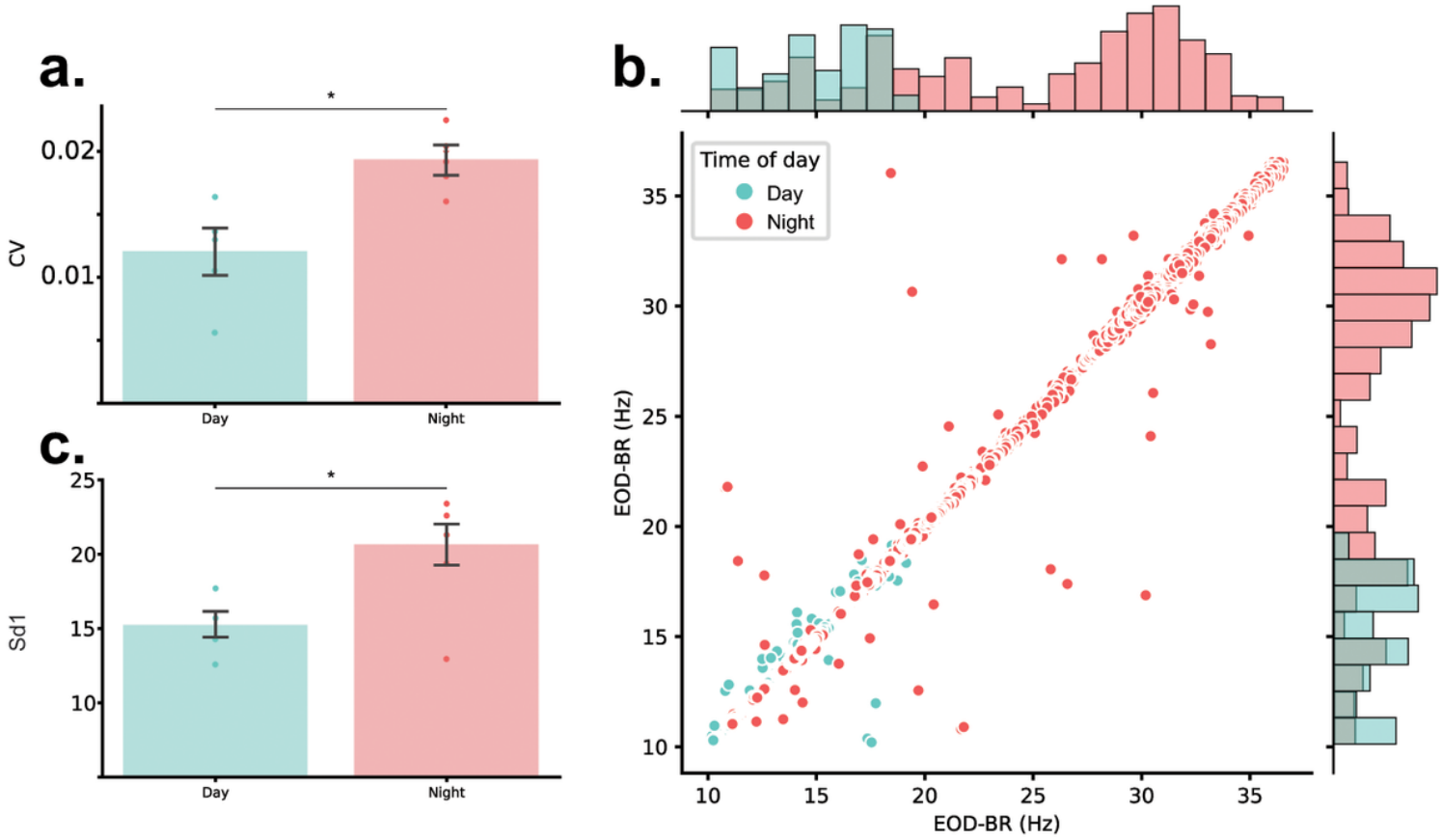

Figure 2

Daily modulations of EOD-BR variability in the natural habitat (n=6) **a.**Coefficient of variation increases significantly at night. **b.** Individual Poincaré plot, day and night values are plotted separately (red and blue dots respectively). Histograms at the margins illustrate frequency's distribution for day and night. **c.** Quantification of Poincaré plots renders the SD1 parameter indicating cycle to cycle variability. SD1 increases at night. *=p≤0.05, **=p≤0.01, ***=p≤0.001, ****=p≤0.0001

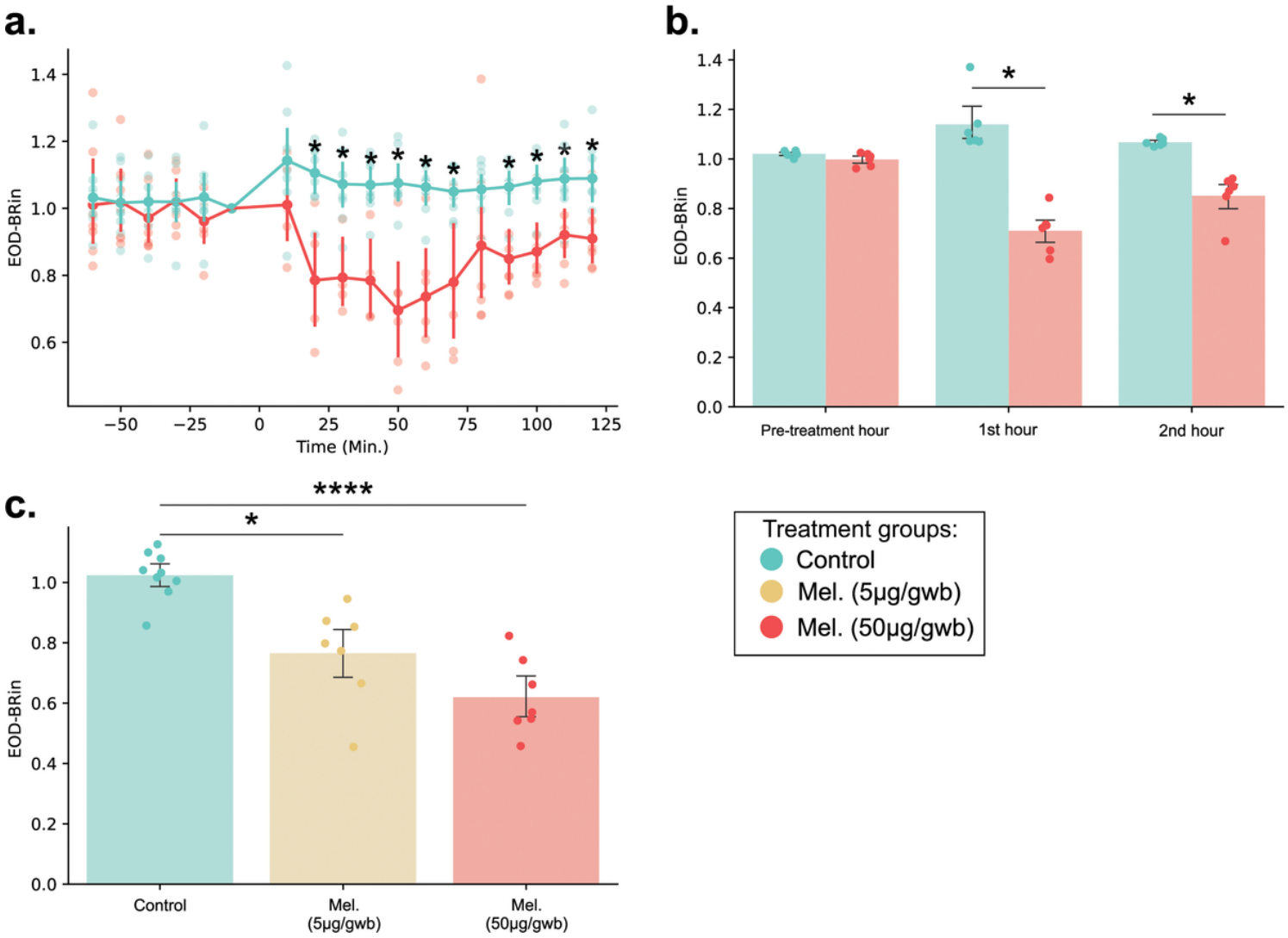

Figure 3

Melatonin effect on EOD-BR. IP melatonin injection led to a decrease in EOD-BR. **a.** EOD-BRin values for melatonin (red, n=7) and control groups (blue, n=9). Dots represent individual EOD-BRin values, lines show mean values and standard deviation. EOD-BRin values in animals treated with melatonin become significantly lower than those of control animals 20 minutes after injection and throughout the whole experiment. **b.** EOD-BRin for melatonin and control groups during the pre-treatment hour and the first and second hour after injection. Melatonin lowers EOD-BR. Indexes are 30% lower during the first hour (Mann-Whitney U test) and 25% lower during the second hour. No differences were found during the pre-treatment hour. **c.** EOD-BRin values at the time of minimum EOD-BR for animals treated with a low (n=7) or high (n=7) melatonin dose as well as control (n=9). The minimum EOD-BRin values were significantly lower in animals treated with either dose compared to control animals. *=p≤0.05, **=p≤0.01, ***=p≤0.001, ****=p≤0.0001

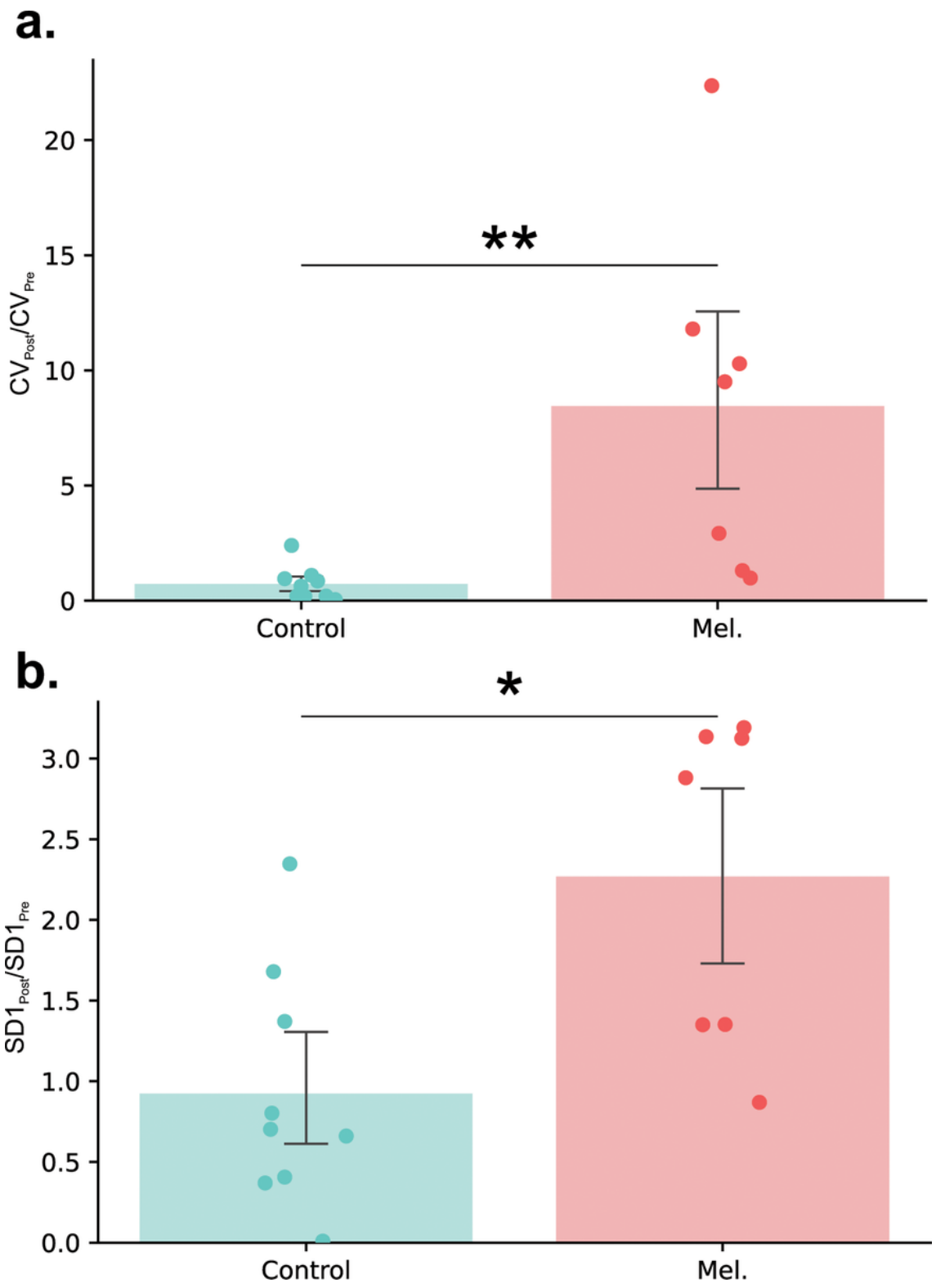

Figure 4

Melatonin effect on EOD-$BR_{var.}$ Melatonin injection caused an increase in EOD-$BR_{var.}$ **a.** Ratio between CV values after (post) and before (pre) treatment with either control vehicle (n=9) or melatonin (n=7). **b.** Ratio between SD1 (cycle-to-cycle variability) values after (post) and before (pre) treatment. *=p≤0.05, **=p≤0.01, ***=p≤0.001, ****=p≤0.0001

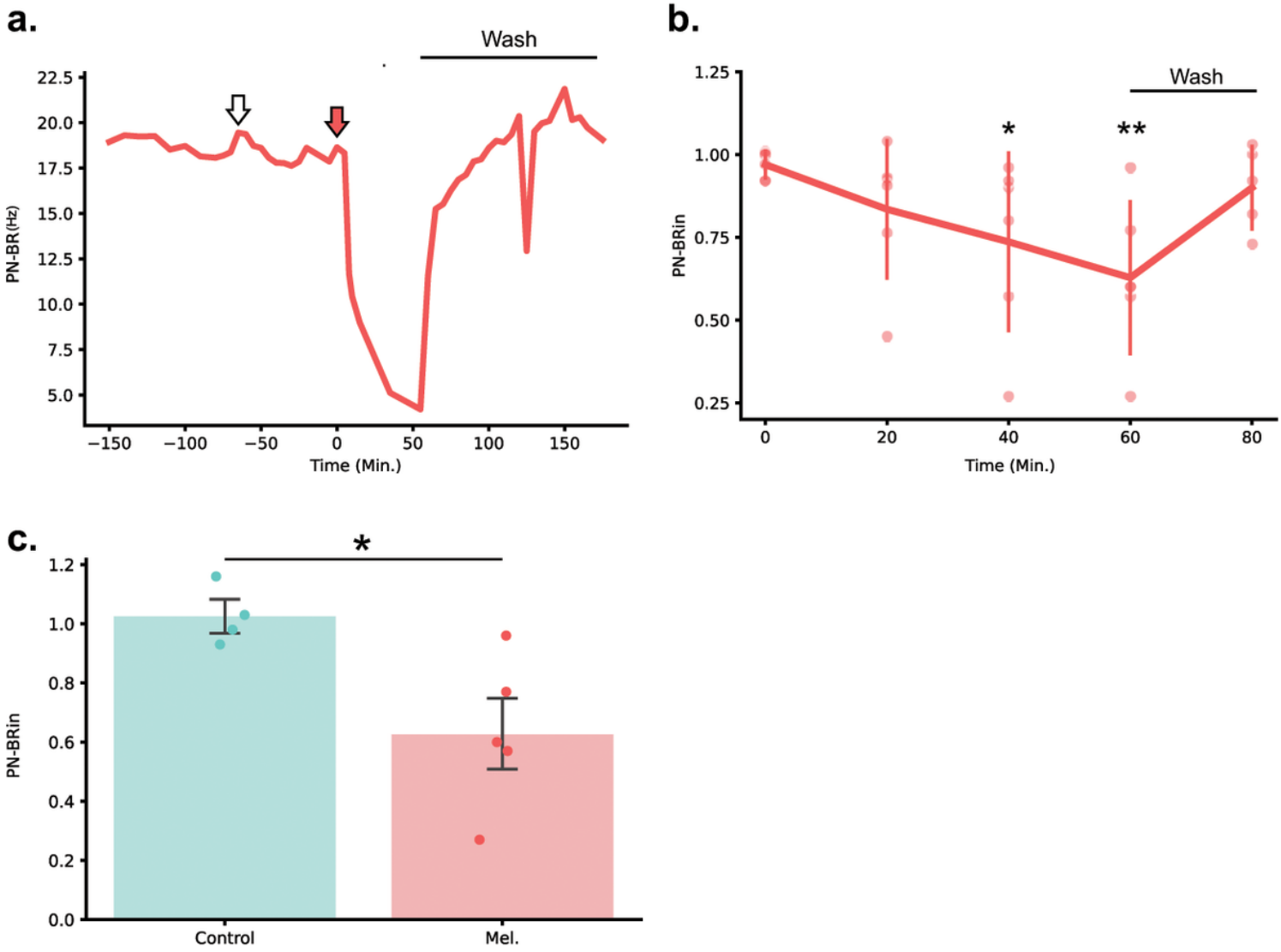

Figure 5

Melatonin effect on the PN's spontaneous activity. Direct administration of melatonin on the PN through perfusion caused a decrease in PN-BR. **a.**Individual example of PN-BR changes after melatonin treatment. The arrows mark the addition of a control solution (white) and a melatonin solution (red). Horizontal line signals washing of the slice. **b.** Mean PN-BRin values (n=6). Time 0 marks the moment of melatonin administration. PN-BRin values were significantly lower 40 and 60 minutes after melatonin administration. The effect was reversed after washing with Ringer solution. **c.** PN-BRin values 60 minutes after treatment with either the control vehicle (n=4) or melatonin (n=6) solution. Control animals showed no change in their PN-BRin values. (*=p≤0.05, **=p≤0.01, ***=p≤0.001).

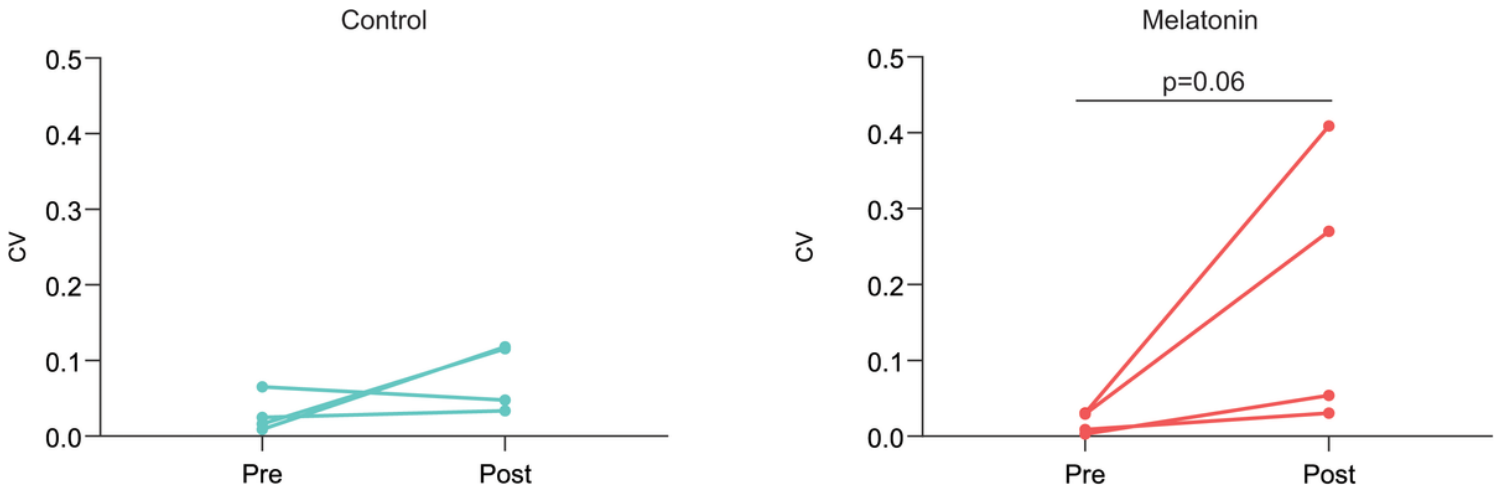

Figure 6

Melatonin effect on the PN-BR_{var}. Direct administration of melatonin on the PN through perfusion appears to cause an increase in the CV of PN-BR. Only 4 out of the 6 slices were stable enough for a variability analysis.