Mammalian retinal Müller cells have circadian clock function

Lili Xu,1 Guoxiang Ruan,1 Heng Dai,1 Andrew C. Liu,2 John Penn,3 Douglas G. McMahon1

1Department of Biological Sciences, Vanderbilt University, Nashville, TN; 2Department of Biological Sciences, The University of Memphis, Memphis, TN; 3Department of Ophthalmology and Visual Science, Vanderbilt University, Nashville, TN

Purpose: To test whether Müller glia of the mammalian retina have circadian rhythms.
Methods: We used Müller glia cultures isolated from mouse lines or from humans and bioluminescent reporters of circadian clock genes to monitor molecular circadian rhythms. The clock gene dependence of the Müller cell rhythms was tested using clock gene knockout mouse lines or with siRNA for specific clock genes.
Results: We demonstrated that retinal Müller glia express canonical circadian clock genes, are capable of sustained circadian oscillations in isolation from other cell types, and exhibit unique features of their molecular circadian clock compared to the retina as a whole. Mouse and human Müller cells demonstrated circadian clock function; however, they exhibited species-specific differences in the gene dependence of their clocks.
Conclusions: Müller cells are the first mammalian retinal cell type in which sustained circadian rhythms have been demonstrated in isolation from other retinal cells.

Our vision is different at different times of day because our retina works differently at different times of day. Daily rhythms in visual function are not just simple responses to the daily light-dark cycle but are the overt expression of an endogenous, self-sustained circadian clock in the retina that drives many rhythms in retinal physiology and metabolism [1]. Numerous aspects of retinal function are under the control of an endogenous retinal circadian clock, including melatonin release [2,3], dopamine synthesis [4,5], gamma-aminobutyric acid (GABA) turnover rate and release [6], extracellular pH [7], electroretinogram (ERG) b-wave amplitude [8], rod disk shedding [9], and circadian clock gene expression [10-12]. In addition, the mammalian retinal clock and its outputs influence the cell survival and growth processes in the eye, including the susceptibility of photoreceptors to degeneration from light damage [13,14], photoreceptor survival in animal models of retinal degeneration [15], photoreceptor and retinal ganglion cell survival in aging [16], and the degree of refractive errors in primate models of myopia [17]. Circadian signals originating in the retina drive rhythms in the hypothalamic biologic clock, even in the absence of light-dark cycles [18].

Although the pervasive influence of the retinal circadian clock is well documented, the cell-specific organization of the mammalian retinal circadian clock is not completely understood. Mapping of the cell-specific expression of the core clock genes that generate circadian rhythms (Bmal1, Clock, Per1, Per2, Cry1 and Cry2) suggests that multiple populations of retinal neurons may be capable of circadian rhythmicity [12,19,20]. Similarly, real-time gene expression imaging of molecular circadian rhythms has demonstrated circadian rhythmicity in the photoreceptor and inner nuclear layers of the retina [11,21,22].

Efforts to date at elucidating the cellular organization of the retinal clock have focused primarily on retinal neurons; however, retinal Müller cells are key glial cells that span the entire depth of the retina and integrate physiology and metabolism across the retinal cell layers [23]. In the central biologic clock of the hypothalamus, the suprachiasmatic nucleus, astrocyte glia express clock genes and exhibit endogenous molecular circadian rhythms [24]. Here, we report that retinal Müller cells express the full set of core clock genes and generate molecular circadian rhythms in isolation from other retinal cell types and that Müller cell circadian clocks have a clock gene dependence unique from the retina as a whole.

METHODS

Müller cell culture: Primary Müller cell cultures were derived from wild-type, Per1−/−, Per2−/−, and Per1−/−/Per2−/− transgenic mouse and human retinas. The Period gene knockout (KO) mouse lines were originally obtained as a gift from D. Weaver [25], backcrossed for more than 12 generations on the C57BL6 background, and then crossed to PER2::LUC bioluminescent reporter mice on the C57 background (a gift of J. Takahashi [26]) to generate Period gene knockout reporter mice [27]. Human Müller cells were isolated from human donor eyes using the method described in Hicks and Courtois (also described in the section on
Experiments assessing the effects of gene

...2016; 22:275-283 <http://www.molvis.org/molvis/v22/275>

...26

To test for the

Purified Müller cells were

To test whether

luminometer (Actimetrics) for recording. The effectiveness

17504-044], and 0.1 mM of luciferin [Promega Cat#E1601,

[52x116] [Sigma-Aldrich Cat#G7513], 2% of B27 [ThermoFisher Cat#

[52x155] DMEM and incubated at 37 °C in 5% CO

Cells were seeded on 35 mm dishes containing serum-free

Medium (ThermoFisher 31-985-062, Grand Island, NY;

two tubes containing 50 µl of Opti-MEM I Reduced Serum

FBS [Sigma F2442-500 ml]), and dissociated with repeated

pipetting using 1 ml. Müller cells from both species were

prewarmed) for 15 min at room temperature; then, siRNA

and Lipofectamine were combined, mixed gently, and

incubated at room temperature for 25 min. Müller cells

expressing siRNA (for mouse or human

Per2, Per1, Per2, Bmal1, or a

control siRNA, Santa Cruz) and Lipofectamine (Thermo-

Fisher Cat#11668-027) for 25 min at room temperature.

Cells were seeded on 35 mm dishes containing serum-free

DMEM and incubated at 37 °C in 5% CO2 and 95% for 6

h, switched to 10% FBS DMEM for 6 h, then changed into

recording medium (medium 199 and 0.7 mM of L-glutamine

[Sigma-Aldrich Cat#G7513], 2% of B27 [ThermoFisher Cat#

17504-044], and 0.1 mM of luciferin [Promega Cat#E1601,

Madison, WI]), and transferred to a Lumicycle multichannel

luminometer (Actimetrics) for recording. The effectiveness

and specificity of the siRNAs were confirmed with quantita-

tive PCR (Appendix 1 and Appendix 2).

Data analysis: Experiments assessing the effects of gene

knockouts or knockdowns were run with two to six inde-

pendent replicates. Effects on rhythmicity were quantified

by analysis using Lumicycle software with the threshold

for considering a condition to be rhythmic being an average

rhythmic power of ≥0.1 and goodness of fit ≥15% [30]. Values

are reported as mean ± standard deviation.

RESULTS

To examine the circadian function of Müller cells, we

first produced primary Müller cell cultures. The cultures

were assessed with double-label immunofluorescence for

4,6-diamidino-2-phenylindole (DAPI), which stains all

nuclei, and glutamine synthetase (GS), a enzymatic marker

specific for Müller cells in the retina (Figure 1A, B). Mergers

of the DAPI and GS staining patterns showed that the cell

cultures were highly enriched for Müller cells (Figure 1C).

Expression of clock genes in Müller cells: To test for the

expression of circadian clock genes in Müller cells, RNA

was extracted from the Müller cells cultures, and reverse

transcriptase PCR was performed for the core circadian

clock genes: Bmal1, Clock, Per1, Per2, Cry1, and Cry2. Each

gene was readily detected (Figure 1D), indicating that Müller

cells as a population express key genetic components of the

circadian clock.

Circadian rhythms of isolated Müller cells: To test whether

Müller cells exhibit circadian clock function, cultures were

isolated from the mice that harbored a bioluminescent circad-

ian reporter gene, PER2::LUC [26]. Retinal whole mounts

explanted from this mouse reporter line exhibit sustained

circadian rhythms in PER2::LUC bioluminescence, allowing

real-time readout of the cycling of the molecular circadian

clock as the intensity of bioluminescence that varies with

the abundance of the PER2::LUC fusion protein [11]. The

PER2::LUC Müller cell cultures were switched from stand-

dard culture medium to bioluminescent recording medium

containing luciferin, and then the bioluminescent activity

recorded. These cultures exhibited robust circadian rhythms

in PER2::LUC expression that persisted for several days

following initiation of recording, and which could be partially

restored in amplitude with subsequent media changes (Figure

2A).

To further test molecular circadian rhythms in isolated

mouse Müller cells, Müller cell cultures from WT C57 mice

were transduced with lentiviral constructs in which either the

Per2 gene promoter or the Bmal1 gene promoter to drive the
expression of firefly luciferase [29]. Similar to the cultures from the PER2::LUC reporter mice, Müller cells transduced with lentiviral Per2::luc and Bmal1::luc exhibited robust circadian rhythms in bioluminescence, which were sustained for days, and then could be partially restored in amplitude with a change to a fresh culture medium (Figure 2B,C).

Clock gene dependence of Müller cell rhythms: In the whole retina, expression of Perl and Bmal1 is necessary for molecular circadian rhythms, whereas expression of Per2 is not [8,27]. Here, we tested for the necessity of these three clock genes in maintaining molecular circadian rhythms in mouse Müller cell cultures. The dependence on Period genes was tested in the mice by isolating Müller cells from Period
gene knockout mice: Per1−/−, Per2−/− and Per1−/−;Per2−/− double-knockout mice [25]. The circadian clock gene activity of the Per1−/− Müller cells was monitored using the PER2::LUC knockin transgene reporter, while the Per2−/− and Per1−/−;Per2−/− double-knockout Müller cells were monitored using lentiviral Per2::luc or Bmal1::luc reporters. Dependence on Bmal1 was tested by isolating retinas and Müller cells from Bmal1 KO mice crossed with PER2::LUC reporter mice. Individual example traces are shown in Figure 3, Figure 4, and Figure 5 while summary analysis of rhythmicity is shown in Table 1.

Knockout of Per1 led to the loss of PER2::LUC rhythms in the whole retina as well as in isolated Müller cells (Figure 3A–C), similar to the strong effect of Per1 knockout on retinal rhythmicity previously reported [27]. Bmal1 knockout retinas and Müller cells also showed severely blunted circadian rhythms (Figure 3D,E), again mimicking the results from the whole retina [27]. Of note, Müller cells derived from mice harboring a Per1::luc transgene reporter [31] did not exhibit circadian rhythms in Per1 activity (Figure 3F). Knockout of Per2 left Müller cell rhythmicity intact, while the double knockout of Per1 and Per2 resulted in arrhythmicity as would be expected from Per1KO alone (Appendix 3).

To test whether acute suppression of the Per1 or Bmal1 clock gene, as opposed to genetic knockout, may also disrupt Müller cell molecular rhythms, experiments were performed using gene knockdowns with siRNA. In control experiments, neither the Lipofectamine transfection reagent itself nor transfection of a control siRNA sequence that had no known target in the mouse genome altered Müller cell circadian rhythms as measured with PER2::LUC bioluminescence (Figure 4A–C). Application of siRNA targeted to Per1, however, produced arrhythmic Müller cell cultures after an initial set of transients, and rhythmicity was restored after the culture medium was changed (Figure 4D). Application of Per2 siRNA also led to the loss of rhythm (Figure 4E), likely due to direct

Figure 3. Mouse Müller cell culture rhythms are dependent on Per1 and Bmal1 genes. A: Per1::luc retinal rhythms. B: Absence of PER2::LUC rhythms from the Per1 KO retina. C: Absence of rhythms in the Müller cell cultures derived from the Per1−/− PER2::LUC mice. D: Absence of rhythms from the whole retina derived from the Bmal1−/− PER2::LUC mice. E: Absence of rhythms from the Müller cells derived from the Bmal1−/− PER2::LUC mice. F: Absence of Per1::luc rhythms in the Müller cell cultures derived from the Per1::luc mice.
suppression of the PER2::LUC reporter, as Per2−/− tested with a Bmal1 reporter did not disrupt rhythmicity (Appendix 3). Application of siRNA for Bmal1 also negated PER2::LUC rhythms in cultured Müller cells, which were restored after the culture medium was changed (Figure 4F). Thus, ongoing expression of Perl and Bmal1 is necessary for intact robust mouse Müller cell molecular circadian rhythms, and the loss of rhythmicity in gene knockouts is unlikely to be due to purely developmental effects.

To test whether human Müller cells also exhibit molecular circadian rhythms, we transduced purified human Müller cell cultures with the Per2::luc or Bmal1::luc constructs delivered by lentivirus as above. Similar to the mouse Müller cells, the human Müller cell cultures exhibited robust circadian rhythms in Per2 and Bmal1 expression as measured by the bioluminescence output (Figure 5A–C). We also examined the gene dependence of these rhythms by siRNA knockdown as above but used the human Perl, Per2, and Bmal1 sequences for targeting. In contrast to the mouse, knockdown of Perl left the human Müller cell rhythms intact, as measured by Per2::luc (Figure 5D), as did knockdown of PER2, measured with Bmal1::Luc (Figure 5E). Knockdown of Bmal1, however, resulted in the loss of rhythms measured with Per2::Luc (Figure 5F).

**DISCUSSION**

Our findings indicate that mammalian retinal Müller cells express the canonical circadian clock genes, are capable of sustained circadian oscillations in isolation from other cell types, and exhibit unique features of their molecular circadian clock compared to the retina as a whole. These are the first mammalian retinal cell type to be shown to exhibit sustained circadian rhythms in isolation from other retinal cell types in purified cell cultures.

A fundamental question regarding the circadian organization of the mammalian retina is which cell types are the loci of endogenous rhythms generation. Previous studies have mapped the coordinate expression of the complete set of
canonical clock genes to all major retinal neuronal subtypes, with the possible exception of rods [12,19,20], and shown that the photoreceptor layer (rods and cones) and the inner retina (horizontal cells, bipolar cells, amacrine cells, ganglion cells, Müller cells) can each express molecular circadian rhythms in isolation [11,21]. In the intact in vivo mouse retina, dopaminergic amacrine cells, nitric oxide synthase (NOS)–positive amacrine cells, and cones have also been shown to express Per1 clock gene rhythms [20,32,33]. These studies suggest that multiple cell types within the retina could be a source of the circadian rhythm, but do not distinguish between endogenously rhythmic versus passively driven cell types.

We have shown that Müller cells produce endogenous circadian rhythms in the cycling of canonical clock genes in isolated purified cell cultures, demonstrating that Müller cells have endogenous circadian clocks that likely contribute to retinal circadian rhythms. Müller cell nuclei reside in the inner nuclear layer (INL) and likely contribute to the high amplitude molecular rhythms observed in that region of the retina with the PER2::LUC reporter transgene [11]. In addition, as glial cells that span the entire depth of the neural retina, Müller cells have the potential to influence many retinal cell types and processes, through their regulation of ion fluxes and neurotransmitter uptake [23]. One of the principal functional circadian rhythms in the retina is in the amplitude of photopic light responses, which can be quantified by the ERG b-wave [34,35]. Through regulation of ionic and neurotransmitter transport, Müller cell circadian function may play a role in the circadian rhythm in retinal light responsiveness.

The overall molecular basis of the mouse Müller cell circadian clock is similar to that of the mouse retina as a whole. Gene knockout and knockdown experiments showed that mouse Müller cell rhythms require expression of the clock gene Per1 or Bmal1, but not Per2, as does the intact retina [27]. However, although Müller cells require Per1 to express circadian oscillations in PER2::LUC bioluminescence, Per1 promoter activation, as read out by Per1::Luc,

Figure 5. Human Müller cells exhibit molecular circadian rhythms that depend on Bmal1. A: Bioluminescence rhythms from the human Müller cells transduced with Per2::luc lentivirus. B: Bioluminescence rhythms from the human Müller cells transduced with Per2::luc lentivirus and control siRNA. C: Bioluminescence rhythms from the human Müller cells transduced with Per2::luc lentivirus and Lipofectamine. D: Bioluminescence rhythms from the human Müller cells transduced with Per2::luc lentivirus and Per1 siRNA. E: Bioluminescence rhythms from the human Müller cells transduced with Bmal1::luc lentivirus and Per2 siRNA. F: Absence of bioluminescence rhythms from the human Müller cells transduced with Per2::luc lentivirus and Bmal1 siRNA.
does not appear to be expressed with a circadian rhythm in mouse Müller cells (Figure 4F). The role of Per1 in mouse Müller cell clocks may be analogous to Period in Drosophila photoreceptors in which the presence of the Per gene is required, but not rhythmic cycling of the promoter, for the generation of molecular circadian rhythms in the fly eye [36]. Per1 cycles in abundance in the mouse retina as a whole but with a somewhat damped amplitude [12], possibly due to non-circadian expression in Müller cells but circadian expression in retinal neurons, including dopaminergic and NOS amacrine cells, and cone photoreceptors [20,32,33].

Human Müller cells also exhibit in vitro circadian rhythms in clock gene expression, as read out by the lentiviral Per2 and Bmal1 bioluminescent constructs. Surprisingly, the rhythm in these cells may not depend on Per1 expression, as molecular rhythms are maintained following the knockdown of Per1 with siRNA in human Müller cells with siRNA. This is distinct from what was observed in mouse Müller cells in which either knockout or knockdown of Per1 led to arrhythmicity. Although no germline knockout can be tested in human Müller cells, Per1 siRNA was approximately twice as effective in humans versus mice, reducing Per1 mRNA levels to ca. 25% of the control in humans versus 50% in mice (Appendix 1, Appendix 2, Appendix3). Thus, human Müller cells may have a decreased dependence in Per1 expression for rhythmicity.

Although our results show clearly that Müller cells as a cell class generate circadian rhythms, our data do not address directly whether individual Müller cells may be autonomous clock cells, or whether they require communication with each other to be rhythmic. Additional experiments, using isolated individual Müller cells, or low-density cultures, instead of the high-density cultures we have used, will be necessary to resolve this question. Carbenoxolone, a blocker of gap junctions, a principal form of Müller cell communication in culture, does not disrupt retinal molecular rhythms [11], suggesting gap junctional communication is not necessary for the generation of retinal circadian rhythms.

Another interpretational limitation of our study is that we cannot rigorously exclude the possibility that the gene knockouts and knockdowns we performed led to the loss of rhythmicity in cell populations but not at the individual cell
level. We consider this possibility unlikely, however, as loss of Per1 or Bmal1 has been shown to result in the loss of single cell as well as population rhythms in cellular clocks from several tissues (e.g., [37]).

In summary, mouse and human retinal Müller cells generate endogenous molecular circadian rhythms in purified cell culture, establishing Müller glia as a candidate clock cell population in the mammalian retina. Further studies may shed light on the role that Müller cells play in the cellular organization of the mammalian retinal circadian clock.

APPENDIX 1. EFFECT OF PER1, PER2 OR BMAL1 SIRNA ON RNA EXPRESSION IN MOUSE MÜLLER CELLS.

To access these data, click or select the words "Appendix 1".

APPENDIX 2. EFFECT OF PER1, PER2 OR BMAL1 SIRNA ON RNA EXPRESSION IN HUMAN MÜLLER CELLS.

To access these data, click or select the words "Appendix 2".

APPENDIX 3. A. BIOLUMINESCENCE RHYTHMS FROM PER2KO MOUSE MÜLLER CELLS TRANSDUCED WITH BMAL1 REPORTER B.

To access these data, click or select the words "Appendix 3". Lack of bioluminescence rhythms from Perl/Per2KO double knockout mouse Müller cells transduced with Bmal1 reporter. Arrows indicate introduction of new culture medium.

ACKNOWLEDGMENTS

Supported by NIH R01EY15815 to DGM, NIH R01EY07533 to JSP, NIH R01NS054794 to ACL, and the Vanderbilt Vision Core P30EY008126.

REFERENCES

1. McMahon DG, Iuvone PM, Tosini G. Circadian organization of the mammalian retina: From gene regulation to physiology and diseases. Prog Retin Eye Res 2014; [PMID: 24333669].

2. Tosini G, Menaker M. The clock in the mouse retina: melatonin synthesis and photoreceptor degeneration. Brain Res 1998; 789:221-8. [PMID: 9573370].

3. Tosini G, Menaker M. Circadian rhythms in cultured mammalian retina. Science 1996; 272:419-21. [PMID: 8602533].

4. Doyle SE, Grace MS, McVor W, Menaker M. Circadian rhythms of dopamine in mouse retina: the role of melatonin. Vis Neurosci 2002; 19:593-601. [PMID: 12507326].

5. Nir I, Haque R, Iuvone PM. Diurnal metabolism of dopamine in the mouse retina. Brain Res 2000; 870:118-25. [PMID: 10869508].

6. Jaliffa CO, Saenz D, Resnik E, Keller Sarmiento M, Rosen-stein RE. Circadian activity of the GABAergic system in the golden hamster retina. Brain Res 2001; 912:195-202. [PMID: 11532436].

7. Dmitriev AV, Mangel SC. Circadian clock regulation of pH in the rabbit retina. J Neurosci 2001; 21:2897-902. [PMID: 11306641].

8. Storch K-F, Paz C, Signorovitch J, Raviola E, Pawlyk B, Li T, Weitz CJ. Intrinsic circadian clock of the mammalian retina: importance for retinal processing of visual information. Cell 2007; 130:730-41. [PMID: 17719549].

9. Teirstein PS, Goldman AI, O'Brien PJ. Evidence for both local and central regulation of rat rod outer segment disc shedding. Invest Ophthalmol Vis Sci 1980; 19:1268-73. [PMID: 7429763].

10. Tosini G, Kasamatsu M, Sakamoto K. Clock gene expression in the rat retina: effects of lighting conditions and photoreceptor degeneration. Brain Res 2007; 1159:134-40. [PMID: 17560558].

11. Ruan G-X, Allen GC, Yamazaki S, McMahon DG. An autonomous circadian clock in the inner mouse retina regulated by dopamine and GABA. PLoS Biol 2008; 6:e249-[PMID: 18959477].

12. Ruan G-X, Zhang D-Q, Zhou T, Yamazaki S, McMahon DG. Circadian organization of the mammalian retina. Proc Natl Acad Sci USA 2006; 103:9703-8. [PMID: 16766660].

13. Organisciak DT, Darrow RM, Barsalou L, Kutty RK, Wiggert B. Circadian-dependent retinal light damage in rats. Invest Ophthalmol Vis Sci 2000; 41:3694-701. [PMID: 11053264].

14. Grewal R, Organisciak D, Wong P. Factors underlying circadian-dependent susceptibility to light induced retinal damage. Adv Exp Med Biol 2006; 572:411-6. [PMID: 17249604].

15. Ogilvie JM, Speck JD. Dopamine has a critical role in photoreceptor degeneration in the rd mouse. Neurobiol Dis 2002; 10:33-40. [PMID: 12079402].

16. Baba K, Pozdnyev N, Mazzoni F, Contreras-Alcantara S, Liu C, Kasamatsu M, Martinez-Merlos T, Strettoi E, Iuvone PM, Tosini G. Melatonin modulates visual function and cell viability in the mouse retina via the MT1 melatonin receptor. Proc Natl Acad Sci USA 2009; 106:15043-8. [PMID: 19706469].

17. Iuvone PM, Tiggges M, Stone RA, Lambert S, Latiy AM. Effects of apomorphine, a dopamine receptor agonist, on ocular refraction and axial elongation in a primate model of myopia. Invest Ophthalmol Vis Sci 1991; 32:1674-7. [PMID: 2016444].

18. Lee HS, Nelms JL, Nguyen M, Silver R, Lehman MN. The eye is necessary for a circadian rhythm in the suprachiasmatic nucleus. Nat Neurosci 2003; 6:111-2. [PMID: 12536213].
19. Dorenbos R, Contini M, Hirasawa H, Gustinich S, Raviola E. Expression of circadian clock genes in retinal dopaminergic cells. Vis Neurosci 2007; 24:573-80. [PMID: 17705893].
20. Liu X, Zhang Z, Ribelayga CP. Heterogeneous expression of the core circadian clock proteins among neuronal cell types in mouse retina. PLoS ONE 2012; 7:e50602-[PMID: 23819207].
21. Tosini G, Davidson AJ, Fukuhara C, Kasamatsu M, Castanon-Cervantes O. Localization of a circadian clock in mammalian photoreceptors. FASEB J 2007; 21:3866-71. [PMID: 17621597].
22. Jaeger C, Sandu C, Malan A, Mellac K, Hicks D, Felder-Schmittbuhl M-P. Circadian organization of the rodent retina involves strongly coupled, layer-specific oscillators. FASEB J 2015; 29:1493-504. [PMID: 25573753].
23. Dowling JE. (1987) The Retina: An Approachable Part of the Brain. Cambridge, MA: Harvard University Press.
24. Prolo LM, Takahashi JS, Herzog ED. Circadian rhythm generation and entrainment in astrocytes. J Neurosci 2005; 25:404-8. [PMID: 15647483].
25. Bae K, Jin X, Maywood ES, Hastings MH, Reppert SM, Weaver DR. Differential functions of mPer1, mPer2, and mPer3 in the SCN circadian clock. Neuron 2001; 30:525-36. [PMID: 11395012].
26. Yoo SH, Yamazaki S, Lowrey PL, Shimomura K, Ko CH, Buhr ED, Siepka SM, Hong HK, Oh WJ, Yoo OJ, Menaker M, Takahashi JS. PERIOD2:LUCIFERASE real-time reporting of circadian dynamics reveals persistent circadian oscillations in mouse peripheral tissues. Proc Natl Acad Sci USA 2004; 101:5339-46. [PMID: 14963227].
27. Ruan G-X, Gamble KL, Risner ML, Young LA, McMahon DG. Divergent Roles of Clock Genes in Retinal and Suprachiasmatic Nucleus Circadian Oscillators. PLoS ONE 2012; 7:e38985-[PMID: 22701739].
28. Hicks D, Courtois Y. The growth and behaviour of rat retinal Muller cells in vitro. 1. An improved method for isolation and culture. Exp Eye Res 1990; 51:119-29. [PMID: 2387332].
29. Ramanathan C, Khan SK, Kathale ND, Xu H, Liu AC. Monitoring cell-autonomous circadian clock rhythms of gene expression using luciferase bioluminescence reporters. J Vis Exp 2012; 67:e4234-[PMID: 23052244].
30. Noguchi T, Wang LL, Welsh DK. Fibroblast PER2 circadian rhythmicity depends on cell density. J Biol Rhythms 2013; 28:183-92. [PMID: 23735497].
31. Herzog ED, Aton SJ, Numano R, Sakaki Y, Tei H. Temporal precision in the mammalian circadian system: a reliable clock from less reliable neurons. J Biol Rhythms 2004; 19:35-46. [PMID: 14964702].
32. Witkovsky P, Veisenberger E, LeSauter J, Yan L, Johnson M, Zhang D-Q, McMahon D, Silver R. Cellular location and circadian rhythm of expression of the biological clock gene Period 1 in the mouse retina. J Neurosci 2003; 23:7670-6. [PMID: 12930806].
33. Zhang D-Q, Zhou T, Ruan G-X, McMahon DG. Circadian rhythm of Period1 clock gene expression in NOS amacrine cells of the mouse retina. Brain Res 2005; 1050:101-9. [PMID: 15978557].
34. Cameron MA, Barnard AR, Hut RA, Bonnefont X, van der Horst GT, Hankins MW, Lucas RJ. Electroretinography of wild-type and Cry mutant mice reveals circadian tuning of photopic and mesopic retinal responses. J Biol Rhythms 2008; 23:489-501. [PMID: 19060258].
35. Jackson CR, Ruan G-X, Aseem F, Abey J, Gamble K, Stanwood G, Palmitter RD, Iuvone PM, McMahon DG. Retinal Dopamine Mediates Multiple Dimensions of Light-Adapted Vision. J Neurosci 2012; 32:9359-68. [PMID: 22764243].
36. Cheng Y, Hardin PE. Drosophila photoreceptors contain an autonomous circadian oscillator that can function without period mRNA cycling. J Neurosci 1998; 18:741-50. [PMID: 9425016].
37. Liu AC, Welsh DK, Ko CH, Tran HG, Zhang EE, Priest AA, Buhr ED, Singer O, Meeker K, Verma IM, Doyle FJ 3rd, Takahashi JS, Kay SA. Intercellular coupling confers robustness against mutations in the SCN circadian clock network. Cell 2007; 129:605-16. [PMID: 17482552].