

Research paper

Prolonged day length exposure improves circadian deficits and survival in a transgenic mouse model of Huntington's disease

Koliane Ouk, Juliet Aungier, A. Jennifer Morton*

Department of Physiology, Development and Neuroscience, University of Cambridge, Cambridge CB2 3DY, United Kingdom

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ABSTRACT

The circadian disruption seen in patients of Huntington's disease (HD) is recapitulated in the R6/2 mouse model. As the disease progresses, the activity of R6/2 mice increases dramatically during the rest (light) period and decreases during the active (dark) period, eventually leading to a complete disintegration of rest-activity rhythms by the age of ~16 weeks. The suprachiasmatic nucleus controls circadian rhythms by entraining the rest-activity rhythms to the environmental light-dark cycle. Since R6/2 mice can shift their rest-activity rhythms in response to a jet-lag paradigm and also respond positively to bright light therapy (1000 lx), we investigated whether or not a prolonged day length exposure could reduce their daytime activity and improve their behavioural circadian rhythms. We found that a long-day photoperiod (16 h light/8 h dark cycle; 100 lx) significantly improved the survival of R6/2 female mice by 2.4 weeks, compared to mice kept under standard conditions (12 h light/12 h dark cycle). Furthermore, a long-day photoperiod improved the nocturnality of R6/2 female mice. Mice kept under long-day photoperiod also maintained acrophase in activity rhythms (a parameter of rhythmicity strength) in phase with that of WT mice, even if they were symptomatic. By contrast, a short-day photoperiod (8 h light/16 h dark cycle) was deleterious to R6/2 female mice and further reduced the survival by ~1 week. Together, our results support the idea that light therapy may be beneficial for improving circadian dysfunction in HD patients.

1. Introduction

Huntington's disease (HD) is a genetic neurodegenerative disorder characterised by motor, cognitive and psychiatric symptoms. It is now well-established that sleep disruption and circadian abnormalities are symptoms associated with HD (for references, see Morton (2013)). Disrupted rest-activity patterns observed in HD patients are recapitulated in multiple HD mice models (Morton et al., 2005; Kudo et al., 2011; Loh et al., 2013; Fisher et al., 2013, 2016). The R6/2 line of HD mice with 250 CAG repeats exhibits a rapid progression in neurological pathology that starts at ~6 weeks of age, and causes an early death at ~22 weeks of age (Wood et al., 2013). The circadian abnormalities appear from the age of 10–12 weeks and result in a complete disintegration of daily cycles by the age of 15–16 weeks (Wood et al., 2013).

The circadian dysrhythmia in R6/2 mice is accompanied at the molecular level by a temporal dysregulation of the clock genes in the suprachiasmatic nucleus (SCN) and clock-dependent genes in peripheral tissues (Morton et al., 2005; Maywood et al., 2010). However, the

molecular machinery in the SCN of arrhythmic 16 week old R6/2 mice remains intact; when the SCN from arrhythmic R6/2 mice were studied *ex vivo*, they expressed normal electrophysiological output and normal endogenous rhythms of circadian gene expression (Pallier et al., 2007). This suggested that rather than being 'broken', the SCN is functionally dysregulated *in vivo* by pathological afferent input. Given that the molecular machinery is intact, the SCN should be able to respond when stimulated appropriately. In support of this idea, it has been shown that pharmacological (Pallier et al., 2007) as well as non-pharmacological interventions (e.g. temporally scheduled feeding (Maywood et al., 2010) and bright light therapy associated with exercise (Cuesta et al., 2014)) are able to improve the rest-activity rhythms of symptomatic R6/2 mice.

In mammals, circadian rhythms are regulated via direct input from retinal photoreceptors to the SCN. Retinal degeneration and functional impairment have been reported in the R6 lines (Helmlinger et al., 2002; Petrasch-Parwez et al., 2004; Batcha et al., 2012; Ragauskas et al., 2014; Ouk et al., 2016), leading possibly to visual and non-visual dysfunctions such as circadian photoreception. However, we have

Abbreviations: ANOVA, analysis of variance; DD, constant darkness; EEG, electroencephalography; HD, Huntington's disease; HPA axis, hypothalamic-pituitary-adrenal axis; LD, light-dark; L-DOPA, levodopa; REM sleep, rapid eye movement sleep; SCN, suprachiasmatic nucleus; WT, wild type

* Corresponding author.

E-mail address: ajm41@cam.ac.uk (A.J. Morton).

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shown that R6/2 mice are able to respond to photic manipulations such as 6 h jet-lag paradigm as well as 23 h-day (with light exposure at 100 lx) (Wood et al., 2013). We have also seen that the photoreceptors responded to bright light therapy at 1000 lx (Cuesta et al., 2014). Therefore, in the present study we manipulated the light/dark (LD) cycles by changing the photoperiod length as a way to modify the rest-activity rhythms of R6/2 mice.

Under the standard 12 h light/12 h dark cycle, R6/2 mice show a breakdown in their rest-activity rhythms from around 15–16 weeks (Wood et al., 2013), accompanied with a progressive general health deterioration and loss of body weight. In this study, we hypothesised that a prolonged daily light exposure would reduce aberrant daytime activity. We tested the effect of a long-day photoperiod (16 h light/8 h dark cycle) on the rest-activity rhythms, body weight and survival of R6/2 mice. For comparison, we also tested R6/2 mice under a short-day photoperiod (8 h light/16 h dark cycle) and a standard photoperiod (12 h light/12 h dark cycle).

2. Material and methods

2.1. Ethic statement

All experiments were carried out in accordance with the UK Animals (Scientific Procedures) Act 1986, and with the approval of the University of Cambridge Animal Welfare and Ethical Review Board.

2.2. Animals and housing conditions

Wild type (WT) and R6/2 mice were taken from a colony established in the University of Cambridge (CBA x C57BL/6J background) and obtained following the breeding strategy used by Prof. Gill Bates group (Mangiarini et al., 1996). The genotype and the number of CAG repeats of each mouse were determined from tail snips by Laragen (Los Angeles, USA) using GeneMapper (Morton et al., 2009) before, and verified after, the experiments. The 72 R6/2 mice used in the whole study had a mean CAG repeat length of 250 ± 1 .

Prior to the experiments, mice were kept in their home cage, with a maximum of 10 animals of the same sex and genotype in each cage. The mice were maintained in a controlled environment with 12:12 light-dark cycle, room temperature of 21–23 °C and humidity of $55\% \pm 10$, and had *ad libitum* access to dry laboratory food and water. All mice were transferred to clean cages once weekly.

During the circadian studies, mice were housed individually in a light-tight and sound-proof Scantainer ventilated cabinet (Scanbur, Denmark) with controlled humidity ($55\% \pm 10$) and temperature (21–

23 °C), and a built-in light system (100 lx). Mice had *ad libitum* access to food and water, which was delivered by lowered bottles with elongated spouts to facilitate access for symptomatic R6/2 mice. The activity of the mice was checked daily and the mice were visually examined twice a week. As an indication of general well-being, mice were weighed once a week throughout the study and then twice weekly once the body weight of R6/2 mice started to decrease.

2.3. Analysis

Activity of the mice in circadian cages was recorded continuously throughout the experiment with motion sensors (Bosch, Germany) placed on top of each cage and connected to a computerised recording system (Clocklab; Actimetrics, Evanston, IL, USA). Total activity data were double plotted in actograms using 5-min bins. All behavioral analyses were performed using Clocklab software. Period length was calculated using a least-square fits regression line using 7 continuous activity onsets. The Chi-squared periodogram function of Clocklab was used to verify strength of rhythmicity using a line of significance at 0.001. Duration of the active period (alpha) was calculated as the difference between the means of the regression lines drawn through the activity onsets and corresponding offsets. The distribution of the general activity during the active and rest periods was determined using the profile activity function of the software. Rest-activity ratios were calculated as the amount of activity occurring during the light period as a fraction of the amount of activity occurring during the nocturnal activity. Phase angle of activity to light onset and offset was calculated to determine the entrainment of the circadian locomotor activity rhythms to the light-dark schedule. For this, we determined mean time of day when mice were starting (or ending) their activity phase using least-square regression lines fitted to the activity onsets (or offsets) of the targeted time span. We then calculated the time difference with local time when lights were on (or off). Time of activity onsets and offsets were also verified from the analysis of the profile activity. Only rhythmic mice were included in the analysis. Acrophase (peak time) in activity rhythms, a parameter of rhythmicity strength, was estimated using Clocklab function by fitting the activity of each day to a sine function with a period of 24 h. For each parameter, data were averaged across 7 consecutive days. General activity during active and rest period was determined using the profile activity function of the software.

2.4. Lighting conditions

All the mice were first placed in the circadian cabinet at 8 weeks of

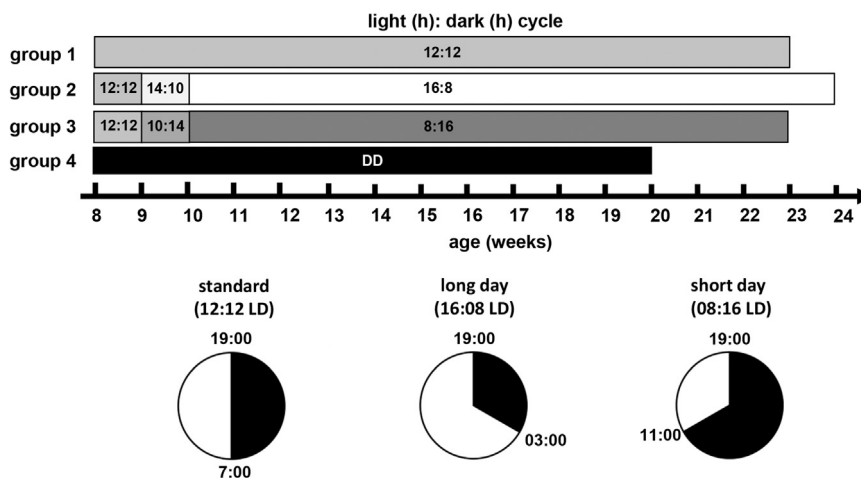


Fig. 1. Experimental time and light schedule for photoperiod testing. Circadian data were collected from mice placed under four different photoperiod lengths: standard condition (12:12 LD cycle), long-day photoperiod (16:8 LD cycle), short-day photoperiod (8:16 LD cycle) and DD (constant darkness). Lights off was set at the same local time of 19:00 for standard, long- and short-day photoperiods.

age under 12:12 LD cycle (lights on at 7 am and off at 7 pm) for 1 week of habituation. After this, the photoperiod was either maintained or changed (Fig. 1), with the light offset remaining at 7 pm.

Three lighting conditions were used. In the first condition (standard), WT and R6/2 mice ($n = 8$ for males and $n = 9$ for females) were maintained under standard light-dark conditions (12:12 LD cycle) for the remainder of the experiment.

In the second condition (long day), at 9 weeks of age, WT and R6/2 mice ($n = 10$ for males and $n = 8$ for females) were placed for one week under 14:10 LD cycle (lights on at 5 am and off at 7 pm), and at 10 weeks of age shifted to a 16:8 LD cycle (lights on at 3 am and off at 7 pm) for the remainder of the experiment.

In the third condition (short day), at 9 weeks of age, WT and R6/2 mice ($n = 8$ for WT male, $n = 10$ for R6/2 male, $n = 9$ for both WT and R6/2 female mice) were placed for one week under 10:14 LD cycle (lights on at 9 am and off at 7 pm), and then at 10 weeks of age shifted to a 8:16 LD cycle (lights on at 11 am and off at 7 pm) for the remainder of the experiment.

Finally, we wanted to compare the effect of light/dark cycles and the effect of constant darkness (DD) on the rest-activity rhythms of R6/2 mice throughout their lifespan. For this, we used and reanalysed a set of data obtained from WT and R6/2 mice placed under DD from a previous published experiment (Cuesta et al., 2012). In that study, WT and R6/2 mice ($n = 9$ for each genotype and each sex) were placed in the cabinet under DD between the ages of 8–20 weeks.

2.5. Survival

Mice were killed when they reached a defined end point, that was when they were considered moribund e.g. when they did not exhibit a righting reflex, or failed to rouse in response to gentle stimulation.

2.6. Statistics

All data are expressed as mean \pm SEM except for the survival curves. Statistical analyses were performed using StatSoft Statistica 19.0 software (version 12, StatSoft Inc., Tulsa, USA) or Prism 5 (GraphPad Software Inc., San Diego, USA). Analysis of variance (ANOVA) with repeated measures was performed to investigate differences between groups, followed by Bonferroni *post hoc* test. Survival data were analysed using the Mantel-Cox Log-rank test. The results were considered significant when $P < 0.05$.

3. Results

3.1. Long-day condition has a beneficial effect on rest-activity rhythms of R6/2 female mice

WT mice were rhythmic throughout the study regardless of the photoperiod condition (Figs. 2A, D, G; 3A, D, G). The rest-activity rhythms of R6/2 mice became progressively disrupted, as expected (Morton et al., 2005; Wood et al., 2013; Cuesta et al., 2014), regardless of the photoperiod under which the mice were placed (Figs. 2B, E, H; 3B, E, H). Nevertheless, the analysis of periodograms showed that exposure to long-day photoperiod delayed the disruption of rest-activity rhythms of R6/2 female mice (Fig. 2C, F, I). Under standard condition and short-day photoperiod, the periodograms showed that amplitude of the rest-activity rhythms of R6/2 female mice decreased dramatically at 14–16 weeks compared to 10–12 weeks (Fig. 2C, I). This decrease in rhythm amplitude was smaller in R6/2 females of 14–16 weeks under long-day photoperiod (Fig. 2F). As for R6/2 male mice, they were already dysrhythmic at 14–16 weeks irrespective of the photoperiod (Fig. 3B, C, E, F, H, I).

3.2. Long photoperiod has a beneficial effect on survival and body weight of R6/2 female mice

The most striking effect of the different photoperiods tested was seen in the survival data of R6/2 female mice (Fig. 4A). Long-day photoperiod significantly extended the lifespan of R6/2 female mice by 2.4 weeks compared to mice placed under standard condition (20.3 ± 0.6 weeks; $P < 0.05$), short-day photoperiod (19.3 ± 0.3 weeks; $P < 0.05$) and DD (20.1 ± 0.6 weeks; $P < 0.05$). By contrast, short-day photoperiod significantly reduced the lifespan in R6/2 female mice compared to standard condition and DD ($P < 0.05$). There was no significant effect of photoperiod length in R6/2 male mice (Fig. 4B).

WT mice grew steadily from 10 weeks of age until the end of the experiment, irrespective of the photoperiod (Fig. 5A, B). By contrast, R6/2 female mice exhibited a decline in body weight from the age of 15 weeks under both standard condition and short-day photoperiod (Fig. 5C). However, weight loss of R6/2 female mice under long-day photoperiod was significantly delayed for ~3 weeks compared to short-day photoperiod and standard condition (age \times genotype effect [$F_{(8,88)} = 11.01$; $P < 0.001$] and [$F_{(9,63)} = 4.70$; $P < 0.001$] respectively). The weight loss of R6/2 male mice started at the age of 13 weeks, irrespective of the photoperiod, and continued until the end of the experiment (Fig. 5D). There were small significant differences when body weight of R6/2 male mice under long-day photoperiod was compared to short-day photoperiod or standard condition, but this is unlikely to be clinically relevant.

3.3. Entrainment to LD cycles is normal in R6/2 mice until the HD-phenotype is advanced

To determine if the beneficial effect of long-day photoperiod on survival was accompanied by changes in rest-activity rhythms, we analysed the circadian parameters of R6/2 mice placed under different photoperiods.

The endogenous period length of R6/2 female mice placed under DD progressively decreased compared to WT mice, with the difference being significant from 12–20 weeks of age ($P < 0.05$, Table 1). When placed under different photoperiods, R6/2 female mice were able to entrain to the new LD cycles, and there were no differences in their period length (~24 h) compared to those of WT mice, until the rest-activity rhythms of R6/2 mice disintegrated (week 17 for short-day photoperiod and weeks 21–22 for long-day photoperiod).

The alpha of female WT and R6/2 mice varied directly according to the time allocated to darkness (Table 2). There was a main effect of photoperiod length on alpha of both WT female [$F_{(3,31)} = 28.11$; $P < 0.001$] and R6/2 female [$F_{(3,19)} = 21.01$; $P < 0.001$] mice. Under DD, the endogenous alpha was ~14 h for both WT and R6/2 mice. Under standard condition and short-day photoperiod, mean alpha for WT mice was of 12.2 ± 0.3 h and 12.7 ± 0.3 h respectively. Under long-day photoperiod, mean WT alpha was of 10.3 ± 0.7 h, with the active phase confined mainly to the 8 hours of darkness. Under the different photoperiod lengths, R6/2 female mice responded the same way as WT mice until the rest-activity rhythms disintegrated. Under long-day photoperiod, we found a main effect of age \times genotype interaction on alpha [$F_{(12,120)} = 13.61$; $P < 0.001$], which was explained by the alpha of R6/2 mice progressively increasing from 10.1 ± 0.4 h at 10 weeks of age up to 14.9 ± 1.1 h at the age of 22 weeks.

To investigate the daily photic synchronisation in R6/2 mice, we analysed the phase angles of entrainment to light onset and offset under each photoperiod condition (Fig. 6). Normal mice entrain to standard light-dark cycle with active period starting when lights are off and ending when the lights are on. Phase angles are parameters to analyse the preciseness of the circadian entrainment. A phase angle is positive when the activity starts before the light cue occurs and is negative when it starts after. We found a significant effect of age \times genotype interaction on phase angles regardless of the photoperiod

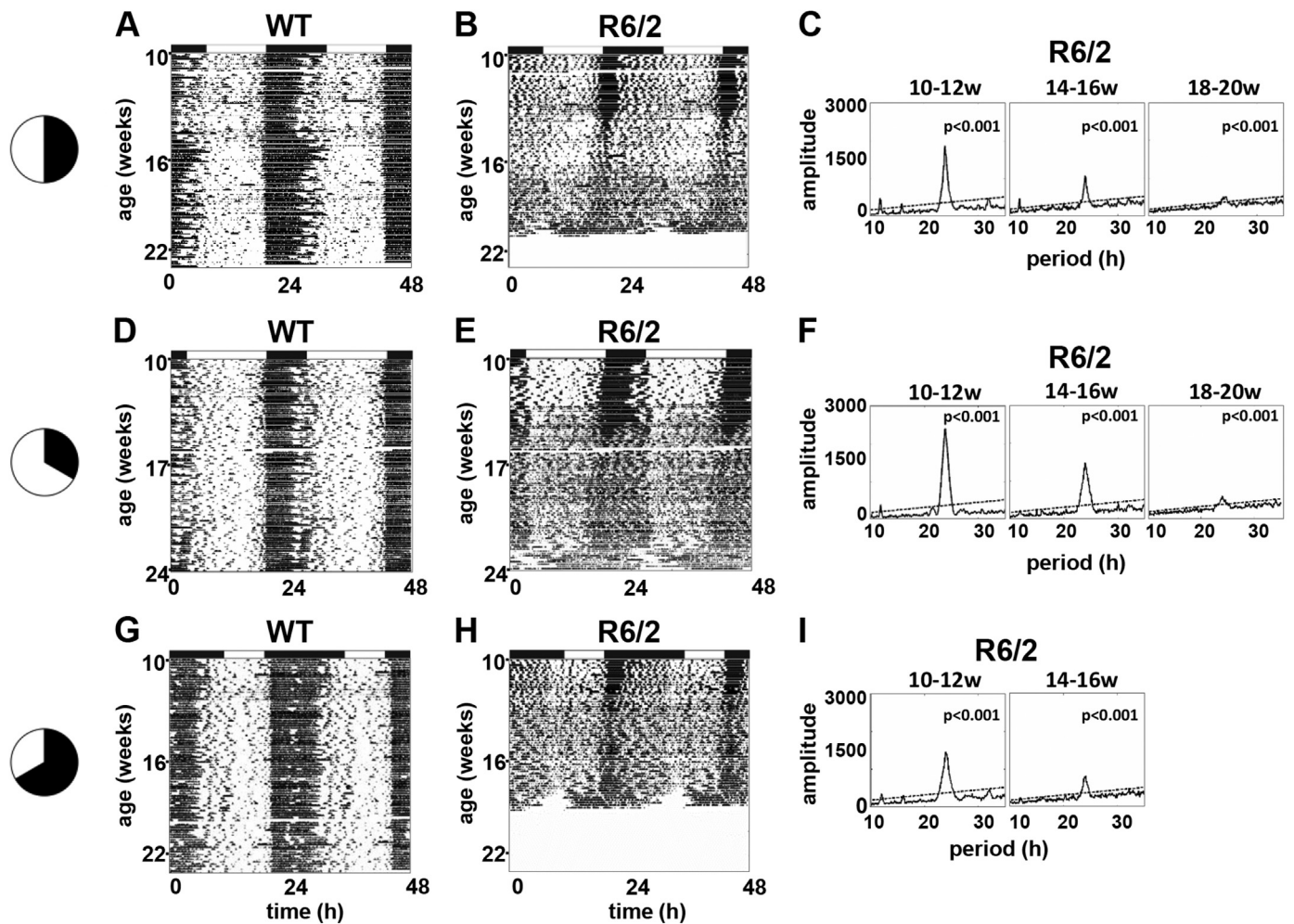


Fig. 2. Disintegration of rest-activity rhythms in R6/2 female mice under different photoperiods. Representative rest-activity profiles show general activity measured in WT (A, D, G) and R6/2 female mice (B, E, H) under standard condition (A, B), long-day photoperiod (D, E) and short-day photoperiod (G, H). Periodograms are shown for R6/2 mice (C, F, I) for each photoperiod, from 10 to 12 weeks (left), 14 to 16 weeks (middle) and 18 to 20 weeks (right). The lines in the periodograms indicate the level of significance ($P < 0.001$). Note the different age axis for D and E compared to A, B, G and H.

length tested. Under standard condition, both WT and R6/2 mice had similar phase angles of entrainment to lights on (Fig. 6A) and lights off (Fig. 6B) until the age of 15–17 weeks when phase angles of R6/2 mice became significantly more positive compared to WT mice ($P < 0.05$). This difference increased with age until the phase angle of synchronisation of the R6/2 mice could no longer be calculated as their rest-activity rhythms had disintegrated.

Under long-day photoperiod, both WT and R6/2 female mice entrained similarly to the light offset until the age of 17 weeks (Fig. 6D), as the phase angle of R6/2 mice was progressively more positive compared to WT mice (+5 h vs. 1 h, $P < 0.05$). Concomitantly, they both had a negative phase angle to light onset, with that of R6/2 female mice becoming significantly different from that of WT female mice from the age the 18 weeks (Fig. 6C). As a result, R6/2 female mice had their active period shifted in the light period.

Under short-day photoperiod, both WT and R6/2 female mice entrained similarly to the light offset (Fig. 6F) until the age of 16 weeks, when the positive phase angle for R6/2 became significantly larger (R6/2 mice, $+2.77 \pm 1.08$ h vs WT mice, -0.85 ± 0.06 h, $P < 0.01$). Both WT and R6/2 mice anticipated the light onset set at 11 am by around 3 hours between 10–17 weeks (Fig. 6E).

The different photoperiod lengths had no significant effect on period length in R6/2 male mice compared to WT mice (Supplementary Table S1). The alpha of R6/2 male mice was similar to that of WT mice under standard condition and short-day photo-

period. Significant differences were only seen in R6/2 male mice under long-day photoperiod who had a longer alpha from the age of 17 weeks compared to WT mice (Supplementary Table S2). R6/2 male mice entrained to the standard condition similarly to the WT mice, until the age of 15–16 weeks when the phase angles became significantly more positive (Supplementary Fig. S1A, B). Under long-day and short-day photoperiods, phase angles of entrainment to light onset was similar between R6/2 and WT male mice (Supplementary Fig. S1C, E). The differences were seen only for the phase angles to light offset when the R6/2 mice anticipated their active period significantly earlier than WT mice from 14 weeks (long-day photoperiod) and 16 weeks (short-day photoperiod) (Supplementary Fig. S1D, F).

In summary, our data showed that R6/2 mice were at first able to entrain their rest-activity rhythms to the LD cycles in a manner similar to that of WT mice, but they progressively anticipated the light offset as their phenotype advanced, by starting their active period significantly earlier than the WT mice.

3.4. Long photoperiod improves nocturnality and rest-activity rhythms in R6/2 mice

We analysed the percentage of activity during the dark period in order to determine the level of nocturnality of R6/2 female mice placed under the different photoperiod lengths (Fig. 7A, C, E). Under long-day photoperiod, we found significant age- and genotype-related changes in

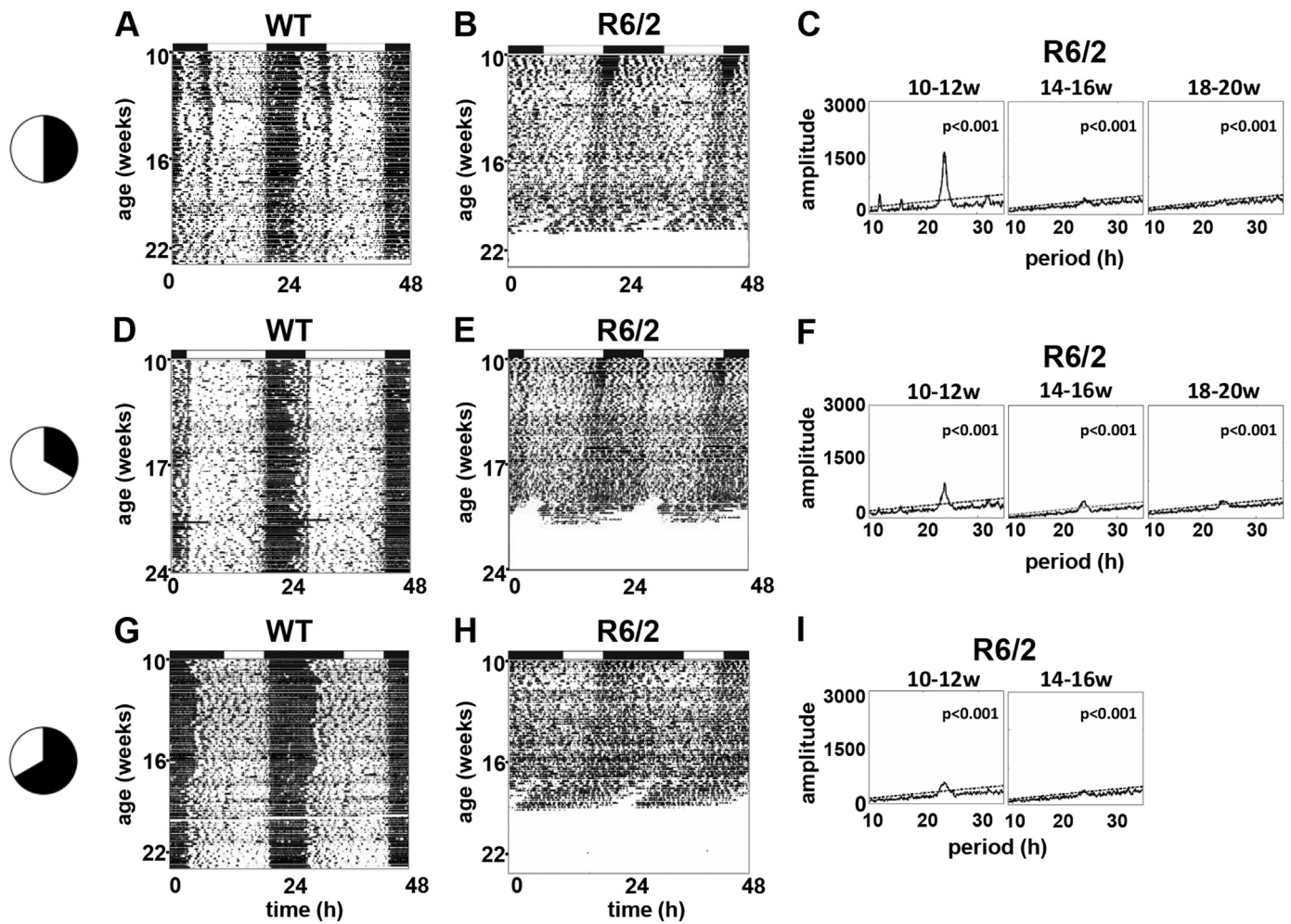


Fig. 3. Disintegration of rest-activity rhythms in R6/2 male mice under different photoperiods. Representative rest-activity profiles show general activity measured in WT (A, D, G) and R6/2 male mice (B, E, H) under standard condition (A, B), long-day photoperiod (D, E) and short-day photoperiod (G, H). Periodograms are shown for R6/2 mice (C, F, I) for each photoperiod, from 10 to 12 weeks (left), 14 to 16 weeks (middle) and 18 to 20 weeks (right). The lines in the periodograms indicate the level of significance ($P < 0.001$). Note the different age axis for D and E compared to A, B, G and H.

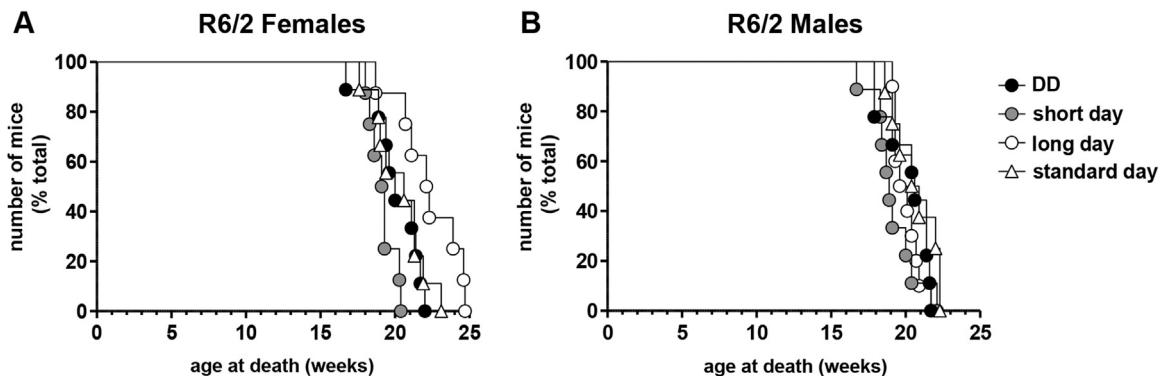


Fig. 4. Beneficial effect of long-day photoperiod on survival of R6/2 female mice. Kaplan-Meier survival curves are shown for R6/2 female (A) and male (B) mice under standard condition (white triangle symbols), long day (white circle symbols), short day (grey circle symbols) and DD (constant darkness, black circle symbols).

the percentage of activity during the dark period [age effect, $F_{(12,144)} = 8.27$; $P < 0.001$; genotype effect, $F_{(1,144)} = 15.40$; $P < 0.01$; Fig. 7C] as well as an age x genotype interaction [$F_{(12,144)} = 4.34$; $P < 0.001$]. Similar effects were seen under standard condition and short-day photoperiod (Fig. 7A, E). While in WT mice the percentage of activity during the dark period was steady throughout the study for each photoperiod ($79.6 \pm 0.1\%$ under standard condition, $66.1 \pm 0.1\%$ under long-day photoperiod and $86.9 \pm 0.4\%$ under short-day photoperiod), the activity of R6/2 mice during the dark period

progressively decreased. *Post hoc* analyses revealed that the differences in R6/2 mice compared to WT mice became significant at 14 weeks of age under short-day photoperiod (Fig. 7E; $P < 0.05$), 18 weeks under standard condition (Fig. 7A; $P < 0.05$), and 20 weeks under long-day photoperiod (Fig. 7C, $P < 0.05$). In order to compare nocturnal behaviour, we looked at the 19 weeks age point: in WT mice, 81% of their activity occurred during the dark period under standard condition, 91% under short-day photoperiod, and 67% under long-day photoperiod. For R6/2 mice, 50% of their activity occurred during

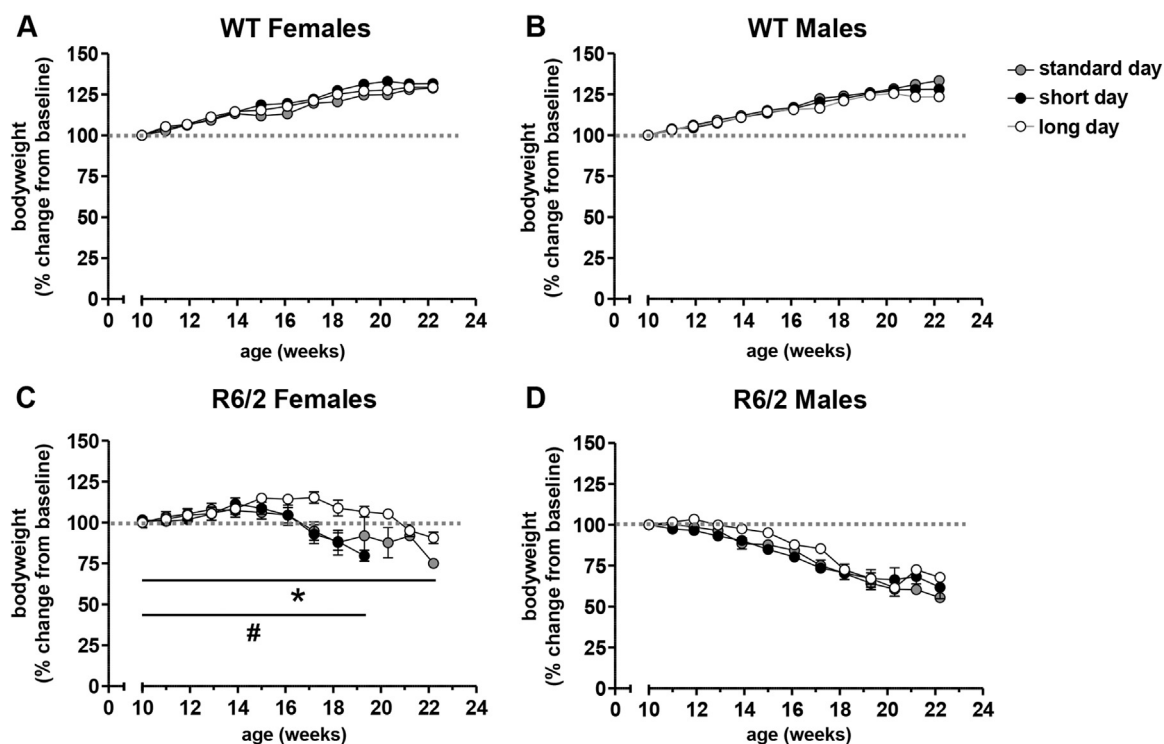


Fig. 5. Long-day photoperiod delays body weight loss in R6/2 female mice. Body weight of WT female (A), WT male (B), R6/2 female (C) and R6/2 male (D) mice are shown from 10 to 23 weeks under standard condition (grey symbols), short-day (black symbols) and long-day (white symbols) photoperiods. Dotted line in each panel shows body weight (100%) at the age of 10 weeks. All data shown are mean ± SEM. Where error bars are not visible, they are obscured by the symbols. Where data are missing from groups, this was because mice were killed at end stage. Asterisks indicate statistical difference between long-day photoperiod and standard condition and hashes between long-day and short-day photoperiods. No statistical difference was found between standard condition and short-day photoperiod. *P < 0.05, #P < 0.05.

the dark period (4.2% per hour of dark) under standard condition, 44% under long-day photoperiod (5.5% per hour of dark) and 54% under short-day photoperiod (3.4% per hour of dark). In R6/2 males, the percentage of activity per hour of dark was similar to R6/2 female mice with 5.5% under long-day photoperiod, 4.1% under standard condition and 4.2% under short-day photoperiod (Supplementary Fig. S2A, C, E).

We analysed the activity ratio (light/dark) of R6/2 female mice placed under the different photoperiod lengths (Fig. 7B, D, F). We found significant age- and genotype-related changes in the activity ratio [genotype effect, $F_{(1,140)} = 16.47$; $P < 0.002$ and age effect, $F_{(14,140)} = 14.16$; $P < 0.001$ respectively] as well as an age x genotype interaction

[$F_{(14,140)} = 12.16$; $P < 0.001$]. Whilst the ratio remained constant over time for the WT mice (0.15 under standard condition, 0.55 under long-day photoperiod and 0.70 under short-day photoperiod), that of R6/2 female mice was at first similar to that of WT mice and then increased progressively; and the difference was significant from the age of 19 weeks (short-day photoperiod, $P < 0.01$), 18 weeks (standard photoperiod, $P < 0.01$), and 17 weeks (long-day photoperiod, $P < 0.01$). The light/dark activity ratio in R6/2 male mice under different photoperiod was similar to that of female mice (Supplementary Fig. S2B, D, F).

Finally, we analysed the acrophase of general activity, as a para-

Table 1
Effect of different daily light exposure on period length of WT and R6/2 female mice.

Age	DD		Short day		Standard condition		Long day	
	WT	R6/2	WT	R6/2	WT	R6/2	WT	R6/2
10 weeks	23.72 ± 0.06 (9)	23.34 ± 0.04 (9)	24.00 ± 0.02 (9)	23.93 ± 0.06 (9)	23.99 ± 0.02 (9)	23.94 ± 0.03 (9)	24.07 ± 0.08 (8)	24.00 ± 0.03 (8)
11 weeks	24.03 ± 0.10 (9)	23.47 ± 0.07 (9)	24.01 ± 0.02 (9)	23.96 ± 0.06 (9)	23.98 ± 0.03 (9)	24.02 ± 0.03 (9)	23.97 ± 0.02 (8)	23.92 ± 0.06 (8)
12 weeks	23.94 ± 0.07 (9)	23.24 ± 0.06 (9)*	24.00 ± 0.02 (9)	23.99 ± 0.04 (9)	23.95 ± 0.04 (9)	23.93 ± 0.02 (9)	23.97 ± 0.02 (8)	23.94 ± 0.05 (8)
13 weeks	24.23 ± 0.09 (9)	23.33 ± 0.05 (9)**	24.00 ± 0.02 (9)	24.02 ± 0.05 (9)	24.00 ± 0.03 (9)	24.02 ± 0.06 (7)	24.00 ± 0.03 (8)	23.98 ± 0.05 (8)
14 weeks	23.86 ± 0.08 (9)	23.1 ± 0.09 (8)	24.00 ± 0.01 (9)	24.02 ± 0.06 (9)	24.00 ± 0.02 (9)	23.92 ± 0.04 (7)	23.97 ± 0.04 (8)	23.93 ± 0.05 (8)
15 weeks	23.92 ± 0.09 (9)	23.22 ± 0.08 (8)**	24.00 ± 0.01 (9)	23.87 ± 0.06 (9)	24.00 ± 0.02 (9)	23.92 ± 0.04 (7)	24.00 ± 0.03 (8)	23.88 ± 0.07 (8)
16 weeks	24.12 ± 0.04 (9)	23.26 ± 0.20 (8)*	24.07 ± 0.02 (9)	23.74 ± 0.1 (9)	24.00 ± 0.03 (9)	23.97 ± 0.06 (6)	24.03 ± 0.03 (8)	23.82 ± 0.07 (8)
17 weeks	24.10 ± 0.05 (9)	23.03 ± 0.12 (8)**	24.01 ± 0.01 (9)	23.81 ± 0.1 (9)**	23.98 ± 0.01 (9)	23.84 ± 0.07 (6)	23.99 ± 0.03 (8)	23.96 ± 0.05 (8)
18 weeks	24.07 ± 0.12 (9)	22.80 ± 0.16 (7)**	23.98 ± 0.03 (9)	24.00 ± 0.15 (6)	23.99 ± 0.03 (9)	23.91 ± 0.07 (5)	24.01 ± 0.04 (8)	23.8 ± 0.03 (7)
19 weeks	23.85 ± 0.05 (9)	22.72 ± 0.11 (2)**	24.04 ± 0.04 (9)		23.96 ± 0.02 (9)	23.99 ± 0.20 (2)	23.99 ± 0.02 (8)	23.83 ± 0.14 (7)
20 weeks	23.89 ± 0.04 (9)	23.21 ± 0.10 (2)*	24.02 ± 0.01 (9)		23.99 ± 0.02 (9)	23.83 (1)	23.99 ± 0.02 (8)	23.96 ± 0.20 (6)
21 weeks			24.01 ± 0.03 (9)		24.04 ± 0.02 (9)		24.08 ± 0.03 (8)	23.63 ± 0.15 (6)**
22 weeks			23.97 ± 0.02 (9)		23.92 ± 0.04 (9)		24.03 ± 0.02 (8)	23.49 ± 0.15 (4)**
23 weeks							24.02 ± 0.04 (8)	23.75 ± 0.57 (2)

*P < 0.05; **P < 0.01.

Table 2
Effect of different daily light exposure on duration of active period of WT and R6/2 female mice.

Age	DD		Short day		Standard condition		Long day	
	WT	R6/2	WT	R6/2	WT	R6/2	WT	R6/2
10 weeks	13.98 ± 0.35 (9)	14.43 ± 0.56 (9)	11.83 ± 0.22 (9)	12.67 ± 0.42 (9)	12.81 ± 0.25 (8)	13.10 ± 0.29 (9)	10.45 ± 0.75 (8)	10.13 ± 0.40 (8)
11 weeks	14.37 ± 0.33 (9)	14.68 ± 0.48 (9)	12.31 ± 0.26 (9)	12.92 ± 0.40 (9)	12.95 ± 0.28 (8)	13.04 ± 0.31 (9)	10.40 ± 0.60 (8)	10.15 ± 0.30 (8)
12 weeks	14.13 ± 0.21 (9)	14.86 ± 0.62 (9)	12.21 ± 0.24 (9)	13.38 ± 0.38 (9)	12.81 ± 0.30 (8)	12.62 ± 0.45 (9)	10.15 ± 0.53 (8)	10.04 ± 0.34 (8)
13 weeks	14.35 ± 0.25 (9)	14.86 ± 0.62 (9)	12.16 ± 0.23 (9)	13.32 ± 0.43 (9)	12.79 ± 0.33 (8)	12.86 ± 0.54 (7)	10.35 ± 0.56 (8)	10.53 ± 0.50 (8)
14 weeks	14.35 ± 0.35 (9)	15.50 ± 0.37 (9)	12.38 ± 0.25 (9)	13.26 ± 0.47 (9)	12.57 ± 0.30 (8)	12.90 ± 0.54 (7)	10.22 ± 0.53 (8)	10.22 ± 0.40 (8)
15 weeks	14.38 ± 0.25 (9)	16.34 ± 0.47 (9)	12.44 ± 0.18 (9)	13.12 ± 0.45 (9)	12.59 ± 0.20 (8)	13.00 ± 0.32 (7)	10.44 ± 0.60 (8)	11.26 ± 0.47 (8)
16 weeks	14.77 ± 0.19 (9)	15.79 ± 0.65 (7)	12.54 ± 0.30 (9)	14.04 ± 0.70 (9)	12.71 ± 0.21 (8)	12.43 ± 0.67 (6)	10.33 ± 0.70 (8)	11.89 ± 0.57 (8)
17 weeks	14.19 ± 0.38 (9)	14.16 ± 0.48 (7)	12.54 ± 0.32 (9)	14.09 ± 0.82 (8)	12.57 ± 0.29 (8)	12.74 ± 0.65 (6)	10.32 ± 0.54 (8)	12.70 ± 1.13 (8)
18 weeks	13.84 ± 0.34 (9)	15.20 ± 0.67 (7)	12.05 ± 0.23 (9)	13.18 ± 1.20 (6)	12.91 ± 0.34 (8)	13.31 ± 0.77 (5)	10.23 ± 0.67 (8)	12.02 ± 0.29 (7)
19 weeks	13.79 ± 0.28 (9)	16.01 ± 0.62 (5)	12.03 ± 0.0 (9)		12.76 ± 0.44 (8)	12.88 ± 0.22 (2)	10.14 ± 0.72 (8)	13.20 ± 0.40 (7)
20 weeks	14.57 ± 0.42 (9)	15.36 (1)	12.08 ± 0.28 (9)		12.77 ± 0.30 (8)	13.75 (1)	10.14 ± 0.75 (8)	13.24 ± 0.64 (6)
21 weeks		16.73 (1)	12.12 ± 0.25 (9)		12.63 ± 0.34 (8)		10.64 ± 0.74 (8)	13.96 ± 0.71 (6)
22 weeks			12.38 ± 0.38 (9)		12.92 ± 0.41 (8)		10.27 ± 0.67 (8)	14.95 ± 1.05 (4)*
23 weeks							10.15 ± 0.65 (8)	17.25 ± 1.26 (3)

*P < 0.05.

meter of rhythmicity strength (Fig. 8). We found age- and genotype-related changes as well as an age x genotype interaction for standard condition (Fig. 8A) and short-day photoperiod (Fig. 8C). For both LD cycles, the activity acrophase of R6/2 female mice progressively occurred closer to the light offset at 7 pm, with significant differences found at 18 weeks (short-day photoperiod, $P < 0.05$) and from 19 weeks (standard condition, $P < 0.01$) compared to WT mice (Fig. 8A, C). Under long-day photoperiod, although we found a main effect of genotype on the acrophase of R6/2 female mice [$F_{(1,10)} = 7.34$; $P < 0.05$], *post hoc* analyses did not reveal any significant differences at any age (Fig. 8B). The activity acrophase of the R6/2 female mice under long-day photoperiod remained 2–3 hours after the light offset, similar to that of WT mice. The analysis of acrophase in R6/2 males showed an important variability and therefore differences with WT mice were not significant until end stage at 20 weeks under long-day photoperiod ($P < 0.01$) and 22 weeks under short-day photoperiod (Supplementary Fig. S3; $P < 0.05$).

In summary, our data suggested that increasing the light period from 12 to 16 h and shortening the dark period from 12 to 8 h forced the R6/2 female mice to be more 'nocturnal', keeping their activity acrophase in the dark period and increasing the percentage of activity in darkness when compared to that of other photoperiods.

4. Discussion

We found that long-day photoperiod prolonged the lifespan of R6/2 female mice by 2.4 weeks, slowed body weight loss and had beneficial effects on the rest-activity rhythms. Long days improved the nocturnality of R6/2 female mice and maintained the acrophase in activity rhythms in phase to that of WT mice, until late symptomatic ages. These effects were not seen in R6/2 male mice tested at the same age.

The prolonged lifespan in R6/2 female mice placed under long-day condition is of particular interest. Although we have identified a number of manipulations that improved neurological function, few of the pharmacological or behavioural manipulations we tried resulted in improved survival in R6/2 mice. For example, chronic treatment with levodopa (L-DOPA) or methamphetamine (0.005% in the drinking water) improved motor abnormalities and rest-activity rhythms in R6/2 mice, but both reduced survival (Hickey et al., 2002; Cuesta et al., 2012). Interestingly, treatment with modafinil/alprazolam (Pallier et al., 2007), or creatine (Ferrante et al., 2000) improved survival in R6/2 mice irrespective of sex while preconditioning treatment with 3-nitropropionic acid (Skillings and Morton, 2016) improved survival only in female mice. It has been shown by others that both exposure to

an enriched environment from an early age, and voluntary physical exercise delays the onset of neurological signs in R6/1 transgenic HD mice (van Dellen et al., 2000, 2008; Pang et al., 2006). In the case of R6/2 mice, we have shown that environmental enrichment improved both neurological function and cognitive performance, and that this behavioural paradigm can have deleterious (Skillings et al., 2014) or beneficial effects (Carter et al., 2000; Wood et al., 2010, 2011) on survival. To our knowledge, there is no other behavioural manipulation shown to improve survival in R6/2 mice. For instance, temporally scheduled feeding and bright light therapy associated with restricted period of voluntary exercise had deleterious or no effect on survival although they improved behavioural abnormalities (Cuesta et al., 2014). It is possible that the long-day photoperiod induces its beneficial effects via an increase in both physical activity in the cage and sensory stimulation. Both of these have a direct and beneficial influence on the morphology of the medium spiny neurons of the striatum (Comery et al., 1996; van Dellen et al., 2000; Spires et al., 2004).

The fact that the effect of long-day photoperiod was seen only on R6/2 female mice is interesting. Studies in transgenic R6/1 HD mice reveal a sexual dimorphism in neuroendocrine (serotonergic and dopaminergic systems), physiological and behavioural endophenotypes, as well as depressive-like behaviours (Renoir et al., 2011, 2014; Du et al., 2015). Interestingly, a sex-dependent delay in onset of circadian phenotype is observed in both R6/2 and BACHD mouse models of HD (Kuljis et al., 2016). Since R6/2 male mice exhibit a circadian disruption earlier than was observed in females, it is possible that the beneficial effects of the long-day photoperiod might have been observed in males if we had started testing them at an earlier age. Indeed, the HD phenotype of male R6/2 mice may have been too advanced at the time we started the experiment to see an effect. The reason that female HD mice develop their circadian phenotype later than male mice is unclear. Several other studies suggest that there are protective mechanisms in female HD mice or rats (Dorner et al., 2007; Bode et al., 2008; Wood et al., 2010). Indeed, there is growing evidence that the role of estrogen is not confined to regulation of ovulation and reproductive behaviour in females of mammalian species (Gillies and McArthur, 2010), but that estrogen also exerts an antioxidant action as a free radical scavenger (Bellanti et al., 2013), with profound effects on learning, memory, mood and neurodegenerative process. Furthermore, there is clinical and basic evidence of a neuroprotective role of estrogen in neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease (McEwen and Alves, 1999; Garcia-Segura et al., 2001; Greenfield et al., 2002; Brann et al., 2007; Garcia-Segura, 2008). In HD patients, there is little evidence of a sex difference in the age at

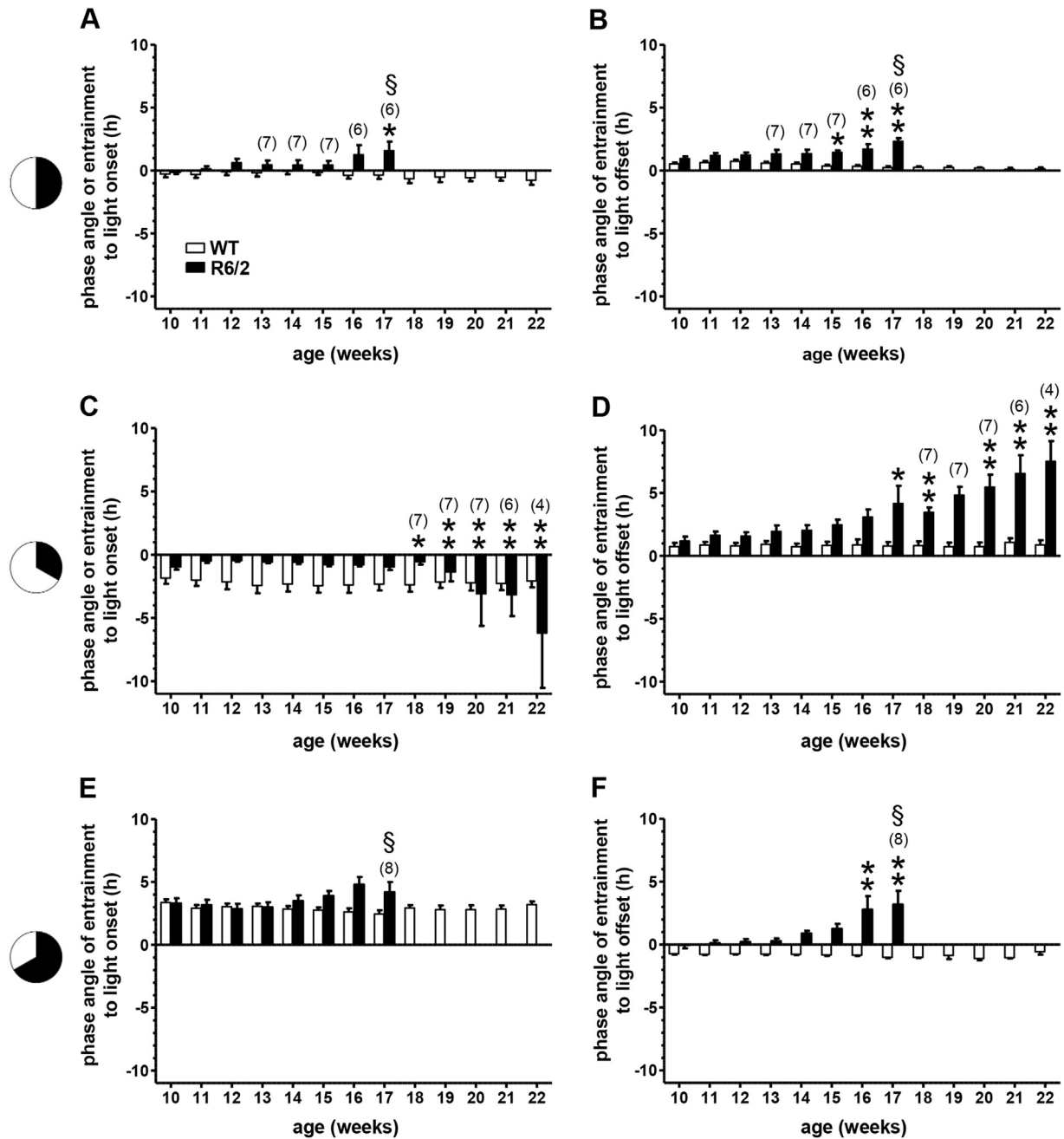


Fig. 6. Phase angles of entrainment are progressively abnormal in R6/2 female mice. Histograms show the phase angle of entrainment to light onset (A, C, E) or offset (B, D, F) for WT (open histograms) and R6/2 (filled histograms) mice placed under standard condition (A, B), long-day photoperiod (C, D) and short-day photoperiod, (E, F). Data were calculated separately and averaged across 7 days and presented as mean \pm SEM. * $P < 0.05$, ** $P < 0.01$. The section sign (§) indicates age after which deaths or arrhythmicity of R6/2 mice made analysis impossible. The number of mice kept under standard conditions was 9 WT and 9 R6/2 mice, under the long-day photoperiod was 8 WT and 8 R6/2 mice and under the short-day photoperiod was 9 WT and 9 R6/2 mice. When numbers of R6/2 mice started to decrease, they are indicated in parentheses in the graphs.

onset or rate of disease progression. However, a study using a transgenic rat model of HD demonstrated that decreased levels of 17 β -estradiol (the most active of the estrogens) correlated with reduced numbers of striatal DARPP32-positive medium-sized spiny neurons in male mice. These neurons are those that are primarily affected in the neurodegenerative process of HD (Bode et al., 2008). This suggests a neuroprotective effect of estrogen in HD.

Under standard and long-day conditions, the negative masking induced by light that suppresses locomotor activity (Mrosovsky, 1999), exerted a pressure on both WT and R6/2 mice to compress their activity (alpha) into the hours of darkness. The entrainment of rest-activity rhythms to the LD cycles by R6/2 mice was progressively less precise as the phenotype advanced, particularly under long-day

photoperiod. The anticipation of active phase compared to lights off seen in R6/2 female mice under long-day photoperiod is likely to be due to the shortening of the endogenous period seen under DD in symptomatic R6/2 mice compared to WT mice. This progressive lack of entrainment to lights off may be explained by a progressive deficit in negative masking and/or a progressive insensitivity to light preventing the R6/2 mice from synchronizing more precisely to the LD cycles. Photoreception occurs in the retina in mammals, via the intrinsically photosensitive retinal ganglion cells that express the photopigment melanopsin, and also via inputs from rods and cones. Retinopathies have been described in R6 lines (Helmlinger et al., 2002; Ragauskas et al., 2014), and we have found that melanopsin and cone opsin expression is downregulated in R6/2 mice as well as in the full length

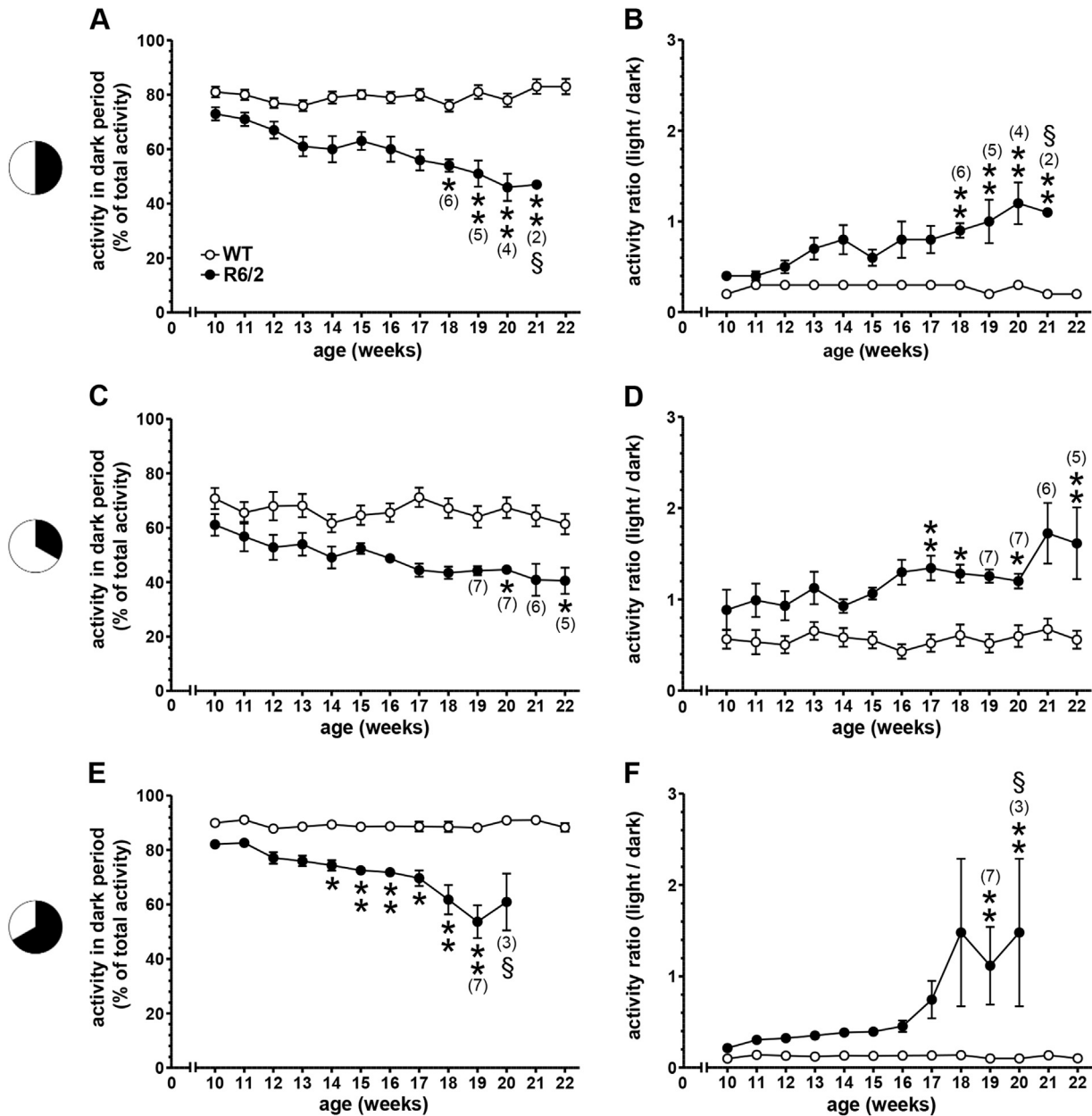


Fig. 7. Effect of photoperiod length is different on general activity parameters of female WT and R6/2 mice. Percentage of nocturnal activity (A, C, E) and light/dark activity ratio (B, D, F) are shown for WT (open symbols) and R6/2 (filled symbols) mice placed under standard condition (A, B), long-day (C, D) and short-day (E, F) photoperiods. Data were averaged across 7 days and presented as mean ± SEM. Where error bars are not visible, they are obscured by the symbols. *P < 0.05, **P < 0.01. The section sign (§) indicates age after which deaths or arrhythmicity of R6/2 mice made analysis impossible. The number of mice kept under standard conditions was 9 WT and 9 R6/2 mice, under the long-day photoperiod was 8 WT and 8 R6/2 mice and under the short-day photoperiod was 9 WT and 9 R6/2 mice. When the numbers of R6/2 mice started to decrease, they are indicated in parentheses in the graphs.

knock-in HD Q175 mice (Ouk et al., 2016), suggesting a progressive deficit in light detection at the level of the HD retina. Therefore, it is possible that a disease-related change in photoreception by the retina contributes to the progressive dysregulation of circadian rhythmicity and entrainment seen in R6/2 mice. We found that increasing the daily light exposure from presymptomatic stage in R6/2 female mice (when the retina was still normal) reinforced the rest-activity rhythms under long-day photoperiod.

The mechanisms underlying the beneficial effect of long day and short night on survival in female R6/2 mice are not known. Since changing the photoperiod length improved the rest-activity rhythms of female R6/2 mice, we speculate that photoperiods may also affect functions such as sleep and metabolism that are circadian-dependent. It has been shown that sleep-wake architecture of R6/2 mice under

standard-day condition becomes particularly aberrant during the dark period (Kantor et al., 2013), with wake amount being dramatically decreased as the symptomatic R6/2 mice progressively lose their ability to maintain long bouts of wakefulness. Concomitantly, both non-rapid eye movement (REM) sleep and REM sleep amounts are doubled in the dark period compared to WT mice. No changes however, were found during the 12 h of light. The SCN has been shown to play a prominent role in the circadian regulation of sleep-wake states (Lee et al., 2009), therefore the increase in REM sleep during the dark period may be caused by a circadian dysregulation. The sleep disruption in R6/2 mice is accompanied by the appearance of a pathological increase in gamma activity (Fisher et al., 2013; Kantor et al., 2013). It would be interesting to determine, in R6/2 mice, whether or not the beneficial and deleterious effects on survival of long-day photoperiod and short-day

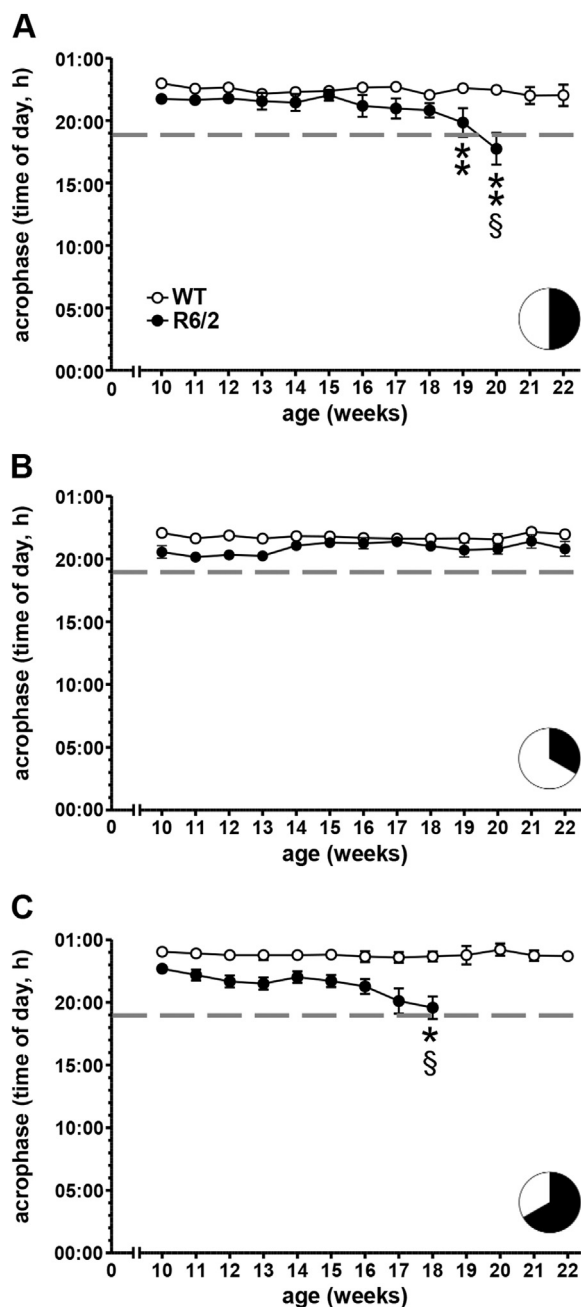


Fig. 8. Long-day photoperiod maintains normal acrophase in activity rhythms in R6/2 female mice. Acrophase is shown for WT (open symbols) and R6/2 (filled symbols) mice placed under standard condition (A), long-day (B) and short-day (C) photoperiods. The grey dashed line indicates the local time for light offset (19:00). Data were averaged across 7 days and presented as mean \pm SEM. Where error bars are not visible, they are obscured by the symbols. * $P < 0.05$, ** $P < 0.01$. The number of mice kept under standard conditions was 9 WT and 9 R6/2 mice, under the long-day photoperiod was 8 WT and 8 R6/2 mice and under the short-day photoperiod was 9 WT and 9 R6/2 mice. The section sign (§) indicates age after which deaths or arrhythmicity of R6/2 mice made analysis impossible. The number of R6/2 mice kept under the standard conditions and included in the analysis progressively decreased at 17 weeks ($n = 7$), 18 weeks ($n = 7$) and 19 weeks of age ($n = 5$), for R6/2 mice kept under the long-day photoperiod at 17 weeks ($n = 7$) and 21 weeks of age ($n = 4$), and under the short-day photoperiod at 18 weeks of age ($n = 6$).

photoperiod, respectively, are reflected in the sleep-wake architecture and the spectral electroencephalography. We speculate that the consolidation of sleep would be improved with long-day photoperiod, as this improved the nocturnality of R6/2 mice.

Disturbances in circadian pacemaking are not restricted to the

brain in R6/2 mice, but also encompass peripheral metabolic pathways (Maywood et al., 2010). In parallel with the circadian disruption, R6/2 mice exhibit a weight loss associated with an increased metabolism, revealed by an increase in oxygen consumption (van der Burg et al., 2008). They also exhibit severe metabolic deficits such as diabetes (Menalled and Chesselet, 2002; Goodman et al., 2008). The improvement in survival in R6/2 female mice placed under long-day photoperiod was accompanied by a delay in body weight loss compared to short-day photoperiod and standard condition. Although body weight does not correlate with survival in the R6/2 mice (Wood et al., 2010), it is a good marker for disease onset and stage of phenotype. Recent studies suggested that photoperiod lengths in normal mice had a significant effect on metabolism (Pyter et al., 2007; Kooijman et al., 2015). For instance, a 16:8 LD cycle prevented the metabolic consequences of circadian disruption via activation of brown adipose tissue (Kooijman et al., 2015), whereas a 8:16 LD cycle had deleterious effects on the hypothalamic-pituitary-adrenal (HPA) axis responsiveness with increases in corticosterone responses to restraint (Pyter et al., 2007). Since metabolism and HPA axis are progressively abnormal in R6/2 mice (Björkqvist et al., 2006; Maywood et al., 2010), it would be interesting to study the effects of long- and short-day photoperiods at a molecular level in R6/2 mice, with particular reference to liver metabolism. The improved survival under long-day photoperiod we observed may be paralleled by delayed metabolic deficits in R6/2 mice.

Circadian abnormalities have been associated with mood disorder such as depression (Landgraf et al., 2016), which is a common psychiatric symptom of HD (Slaughter et al., 2001; Paulsen et al., 2005; Julien et al., 2007). R6/2 mice also develop depressive and anxiety-like behaviours (Ciamei et al., 2015). Bright light therapies have been shown to be beneficial in the treatment of depression (Golden et al., 2005; Pail et al., 2011) as well as circadian disruption in Parkinson's (Willis and Turner, 2007) and Alzheimer's disease (Dowling et al., 2007). Bright light was an efficient intervention to improve rest-activity rhythms of R6/2 mice (Cuesta et al., 2014). In our current study, we found that increasing the period of light exposure at 100 lx from 12 to 16 h was as beneficial to R6/2 female mice as increasing the light intensity during 12 h at 1000 lx. This also supports the idea that the behavioural circadian abnormalities are not only due to photoreception deficit but also to a circadian 'brain' deficit.

In conclusion, we found that a prolonged day length exposure at low light levels from a presymptomatic age had beneficial effects on both survival and on the strength of the circadian rhythmicity in R6/2 female mice. Therefore, photoperiod length seems to be a powerful way to regulate the rest-activity rhythms of R6/2 female mice, when modulated at an appropriate stage of the disease. These findings support the idea that if the length of daily exposure to light is regulated in HD patients, this may help with the treatment of circadian abnormalities.

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Supplementary material

Supplementary data associated with this article can be found in the online version at doi:<http://dx.doi.org/10.1016/j.nbscr.2016.11.004>.

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