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Current Biology

Environmental 24-hr Cycles Are Essential for Health

Highlights

- Long-term exposure to LL impairs rhythms in the central clock
- Muscle strength, bone structure, and immune function are reduced by LL exposure
- Robust environmental rhythms can rescue major health parameters

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In Brief

Lucassen et al. describe that exposure to continuous light, commonly present in intensive care units, chronically attenuates internal 24-hr rhythms leading to a deterioration in muscle strength, bone microstructure, and innate immune response. Upon re-exposure to robust light-dark cycles, these health parameters restore.

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Environmental 24-hr Cycles Are Essential for Health

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SUMMARY

Circadian rhythms are deeply rooted in the biology of virtually all organisms. The pervasive use of artificial lighting in modern society disrupts circadian rhythms and can be detrimental to our health. To investigate the relationship between disrupting circadian rhythmicity and disease, we exposed mice to continuous light (LL) for 24 weeks and measured several major health parameters. Long-term neuronal recordings revealed that 24 weeks of LL reduced rhythmicity in the central circadian pacemaker of the suprachiasmatic nucleus (SCN) by 70%. Strikingly, LL exposure also reduced skeletal muscle function (forelimb grip strength, wire hanging duration, and grid hanging duration), caused trabecular bone deterioration, and induced a transient pro-inflammatory state. After the mice were returned to a standard light-dark cycle, the SCN neurons rapidly recovered their normal high-amplitude rhythm, and the aforementioned health parameters returned to normal. These findings strongly suggest that a disrupted circadian rhythm reversibly induces detrimental effects on multiple biological processes.

INTRODUCTION

> Secundum naturam vivere.—Seneca (*Letters to Lucilius, Letter 5*)

Virtually all organisms have measurable circadian rhythms that help them anticipate and adapt to the environmental day-night cycle. In mammals, these circadian rhythms are orchestrated by neurons within the suprachiasmatic nucleus (SCN), which is located in the anterior hypothalamus. The SCN conveys temporal information to peripheral tissue oscillators, thus producing synchronized circadian rhythms in many bodily processes, including muscle function, bone metabolism, and immune system function [1–4]. Under evolutionary pressure, the circadian system evolved as a robust mechanism for adapting to life in a cyclic environment. Thus, we hypothesize that organisms require clear external cycles in order to maintain a healthy state and that absence of external rhythmicity is detrimental for health.

The use of artificial lighting in modern society—particularly during the night—disrupts the natural robust environmental cycle and is a risk factor for frailty [5]. Nowadays, 75% of the world's population is exposed to light during the night [6]. Moreover, the prevalence of shift work is relatively high around the globe; approximately 20% of workers in Europe, 29% of Americans, and 36% of Chinese and Koreans are engaged in shift work [7, 8]. Importantly, epidemiological studies of shift workers revealed increased prevalence of breast cancer [9], metabolic syndrome [10], osteoporosis [11], and bone fractures [12] in this population. In addition, individuals who are exposed to more light at night tend to have decreased sleep quality [13], increased body weight [14], and a higher prevalence of cardiovascular disease [15]. Although these studies suggest a correlation between artificial light exposure and health, they cannot determine whether this relationship is causal. Animal studies have shown that aberrant light exposure can affect both the immune system [16–18] and metabolic function [19, 20]. However, in these studies, the exposure to light was relatively brief; therefore, the results cannot be translated directly to humans, who are often chronically exposed to disruptions in circadian rhythm.

To test whether long-term exposure to an aberrant light-dark (LD) cycle affects these health parameters, and to test whether these effects are reversible, we exposed mice to continuous light (LL) for 24 weeks, followed by 24 weeks in a standard LD cycle. To measure rhythmicity in the central clock, we performed in vivo electrophysiological recordings in the SCN of freely moving mice implanted with stationary electrodes. Although short-term exposure to LL has been reported to reduce SCN rhythmicity [21–23], whether the effects of long-term LL exposure are chronic has not been studied previously. This issue is particularly important, as the SCN is highly plastic and can adapt to changes in photoperiod, even after exposure for 3 weeks or longer [24]. We measured the effect of long-term LL on skeletal muscle function, bone microstructure, and immune system function at various

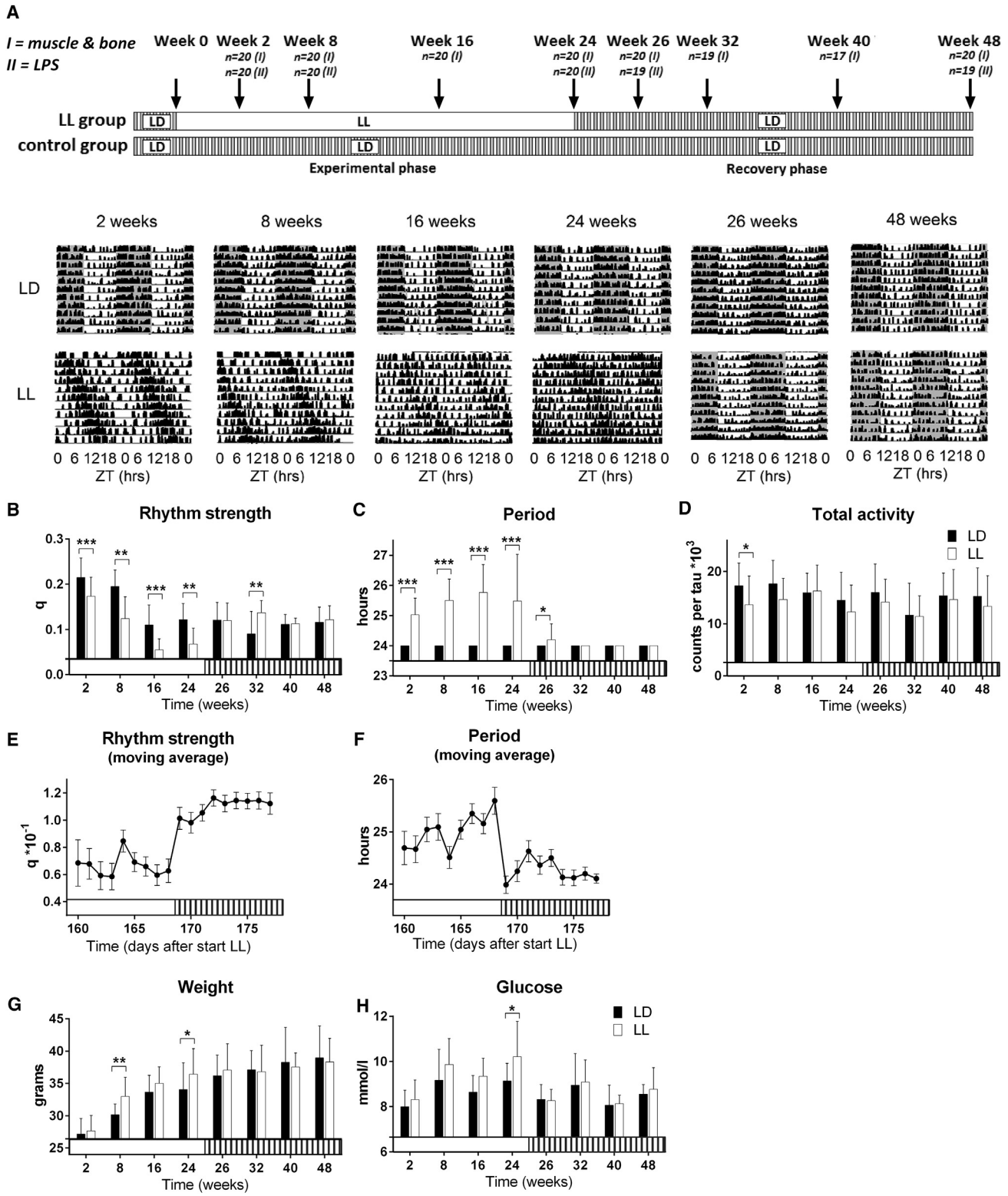

Figure 1. Continuous Exposure to Light Reversibly Disrupts Circadian Rhythmicity in Behavior and Leads to Mild Weight Gain

The top panel shows the experimental protocol. Mice in the LL group were exposed to light continuously from weeks 0 to 24 (experimental phase), then returned to a standard 12 hr light/12 hr dark (LD) cycle from weeks 24 to 48 (recovery phase). Mice in the control group were maintained under the standard LD cycle for the entire 48 weeks. At the indicated time points, mice were sacrificed for muscle and bone experiments or for LPS experiments. Note that separate cohorts of mice were used at every time point. Mice used for the in vivo recordings are not included in the figure.

(legend continued on next page)

time points during and following 24 weeks of LL exposure. Our results support the hypothesis that long-term exposure to LL conditions has significant detrimental effects on a wide range of relevant health parameters. Moreover, the majority of these parameters rapidly returned to normal upon restoring the LD cycle. Thus, our results provide compelling evidence that an absence of environmental rhythmicity plays a causal role in susceptibility to disease.

RESULTS

Reduced Behavioral Rhythms in LL

Wild-type mice (n = 134) were exposed to LL for 24 weeks ("experimental phase"), followed by a 12 hr:12 hr LD cycle for 24 weeks ("recovery phase"). As a control group, a separate set of age-matched mice (n = 119) were exposed to an LD cycle for the entire 48 weeks (Figure 1). Although the strength of the circadian rhythm decreased with age in both the LL and LD groups, this effect was significantly greater in the LL mice compared to the control mice (Figures 1A and 1B). At 2, 8, 16, and 24 weeks, the behavioral rhythm in the mice in the LL group was 19%, 36%, 50%, and 44% smaller, respectively, compared to the control group. At 2, 8, 16, and 24 weeks in LL, 0/22, 1/20, 2/10, and 2/22 mice were arrhythmic as examined by F periodogram, respectively. Rhythm strength of mice that were classified as "rhythmic" by F periodogram analysis was severely dampened (Figure S1). 2 and 8 weeks after returning to the LD cycle, rhythm strength had increased by 79% ($p < 0.001$) and 104% ($p < 0.001$), respectively, compared to rhythm strength at 24 weeks in LL. The period of behavioral rhythm was approximately 25.5 hr in the LL group, did not change over time, and recovered to 24 hr during the recovery phase (Figure 1C). Mice in the LL group had a slight but significant decrease in activity after 2 weeks in continuous light; however, activity levels did not differ significantly between the two groups at any other time point (Figure 1D). Upon returning to a standard LD cycle, the mice in the LL group rapidly recovered in terms of both rhythm period and rhythm strength (analyzed using the moving averages method; Figures 1E and 1F). With respect to metabolism, the mice in the LL group were significantly heavier at 8 and 24 weeks than age-matched mice in the control group (respectively 2.8 and 2.4 g heavier). Similarly, unfasted glucose levels were significantly higher in the mice in the LL group at 24 weeks (Figure 1H). Both the differences in weight and glucose levels disappeared after the LL mice were returned to a standard LD cycle (Figures 1G and 1H).

LL Exposure Attenuates Neuronal Rhythms in the Central Clock

At t = 0 (i.e., baseline), the multiunit activity (MUA) recordings revealed high-amplitude rhythms, with higher levels of electrical activity during the subjective day than during the subjective night (Figure 2A). When exposed to LL, this amplitude decreased initially to 63% of the baseline amplitude (Figures 2A and 2B); at 8 and 24 weeks, the amplitude was reduced further to 34% and 30% of baseline, respectively. The strength of the behavioral rhythm was strongly correlated with MUA amplitude ($R^2 = 0.754$, $p < 0.001$, Pearson correlation), and fluctuations in the strength of the SCN rhythm within individual animals occurred in parallel with fluctuations in the strength of the animal's behavioral activity rhythm. These changes in the SCN's rhythm amplitude recovered rapidly upon shifting back to a standard LD cycle. Importantly, this recovery was mediated primarily by a reduction in the SCN's firing rate in the dark period. Proper positioning of the microelectrode in the SCN was confirmed histologically (Figures 2C and S2).

Skeletal Muscle Function Declines in Animals Exposed to LL

Performance in all three functional muscle tests declined over time in both groups of mice. During the experimental phase, the mice in the LL group performed significantly worse in grip strength and grid hanging duration at every time point compared to the mice in the control group; performance in the wire hanging test was also worse in the LL mice, with significantly different values measured at 8 weeks (Figures 3A–3C). The difference in skeletal muscle function between the two groups disappeared in the recovery phase. Additional analyses in which the results were corrected for body weight and behavioral activity were performed for all measures (grip strength, wire hanging and grid hanging time), and this did not alter effect sizes or significance levels.

Both groups of mice exhibited increased fatigue-related behavior (e.g., distance walked, behavioral intensity, and time spent rearing) and anxiety-related behavior (e.g., time spent in a corner) after completing the tests as analyzed by video analysis; however, these measures did not differ significantly between the LL and control groups (Figure S3). These observations suggest that the effect of LL exposure cannot be attributed to a difference in motivation or anxiety.

We found no difference between the two groups with respect to absolute creatine kinase (CK) levels, the pre-test or post-test CK ratio, or the change in CK levels, indicating that LL exposure did not induce muscle damage. Although the relative amount of fibrosis in the quadriceps muscle was significantly higher in LL mice compared to control mice at 24 weeks (percentage of collagen 5% ± 1% versus 4% ± 1%, respectively), this difference was too small to account for the observed differences in muscle function. We found no significant difference in the mRNA levels of macrophage (*Lgals3*, *CD68*), fibrosis (*Col1a*), regeneration (*MyoG*), mitochondrial biogenesis (*PGC1α*), or fiber type (*Myh7*, *Myh2*, and *Myh4*, which are expressed in type 1 slow

(A) Examples of actograms recorded in LL and control (LD) mice at the indicated time points. Each horizontal row represents behavioral activity measured using a passive infrared motion detector on a double-plotted 24-hr day. Gray background represents the dark period, and white background represents the light period.
(B–D) Rhythm strength, rhythm period, and total activity of mice in the LL and control groups at the indicated times (mean ± SD; n = 8–22 mice per group).
(E and F) 5-day moving averages of rhythm strength and period in behavior in the final days of LL (or control) and in the first days after returning to an LD cycle (mean ± SEM; n = 22 per group).
(G and H) Body weight and unfasted glucose levels in the LL and control groups at the indicated times (mean ± SD; n = 8–10 per group).
The data were analyzed using a two-way ANOVA followed by post hoc least significant difference (LSD). *p < 0.05, **p < 0.01, ***p < 0.001. See also Figure S1.

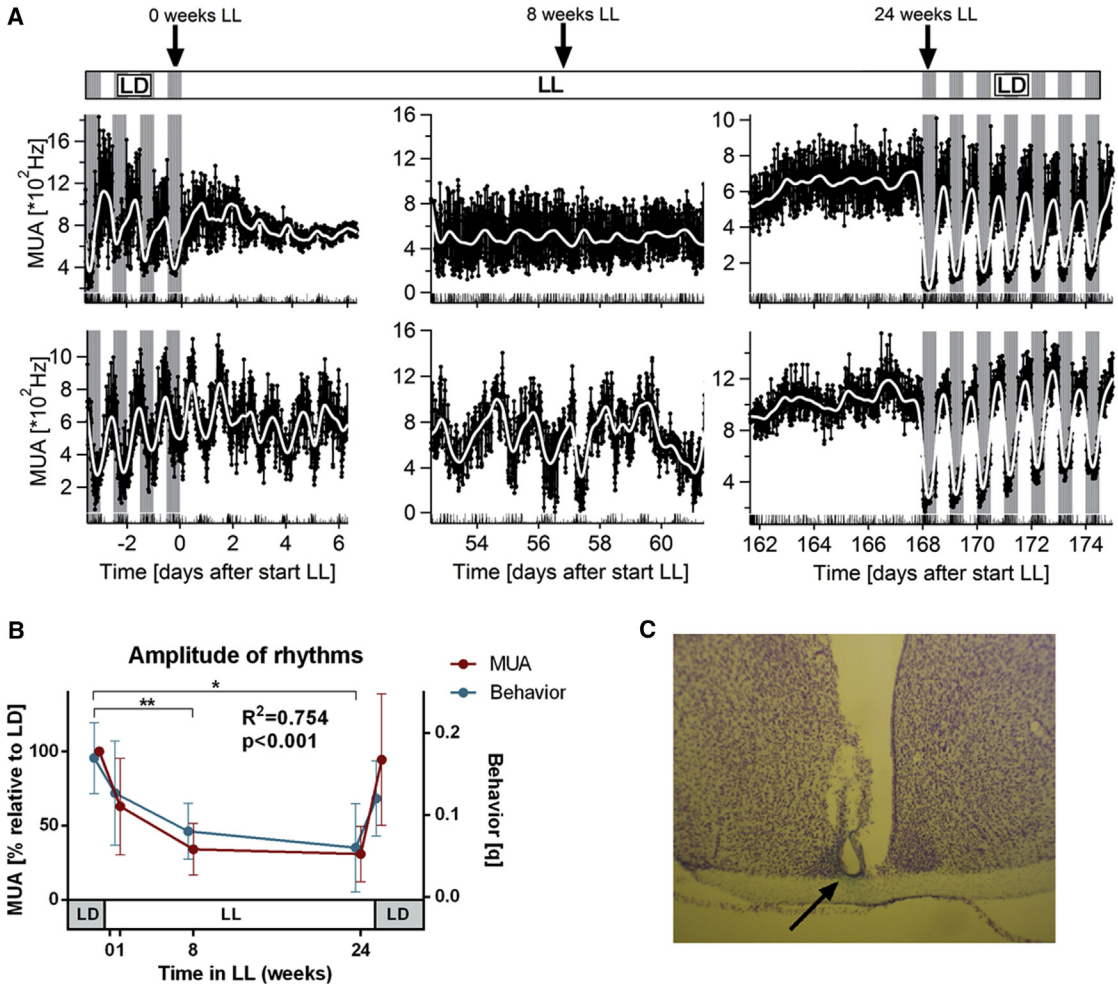

Figure 2. Continuous Exposure to Light Attenuates Rhythmic Neuronal Activity in the Central Clock

In vivo recording electrodes were implanted in the SCN, and multiunit activity (MUA) was recorded in freely moving mice.

(A) Examples of neuronal MUA rhythms recorded in the SCN of two LL mice at the times indicated. Note the more severe loss of rhythmicity in the mouse in the upper row. Gray bars indicate darkness. Behavioral activity is depicted as vertical upticks at the bottom of each graph.

(B) Summary of the amplitude of the neuronal rhythm relative to the amplitude at baseline (red) and the strength of the behavioral rhythm of the same mice (blue) (mean ± SD).

(C) Example of SCN histology with cresyl violet staining. The arrow indicates the location of the electrode. The third ventricle separates the two SCNs that are embedded in the optic chiasm.

Pearson correlation and repeated-measures ANOVA, followed by post hoc LSD. *$p < 0.05$, **$p < 0.01$. See also Figure S2.

fibers, type 2A fibers, and fast type 2B fibers, respectively) markers in quadriceps muscles at 8 and 24 weeks.

Exposure to LL Induces Features Characteristic of Early Osteoporosis

After 8 weeks in the experimental phase, both groups of mice had normal bone maturation and reached their peak relative bone volume density (BV/TV) at 20 weeks of age (Figures 4A and S4). After 8 weeks of LL exposure, the metaphysis and diaphysis of the femurs had a thicker cortex compared to controls (Figure S4). Over the subsequent weeks, the volume, thickness, number, and separation of the trabeculae, the structural model index (SMI), and BV/TV began to differ between the two groups (Figure S4). At the end of the 24-week experimental phase, the trabeculae of the mice in the LL group were 34% smaller in volume and 10% thinner (Figures 4B, 4C, and S4). The mice in the LL group also had 28% fewer trabeculae, which were separated more (by 16%). The trabeculae in this group were also more rod-like in shape compared to the control group. Together, these findings are characteristic of the early stages of osteoporosis.

In the LL group, cortical bone thickness in the metaphysis was increased by 5%; however, LL exposure had no effect on cortical volume in the metaphysis or any cortical parameters in the diaphysis (Figures 4B, 4C, and S4). Remarkably, bone structure no longer differed between LL-exposed mice and controls after returning the mice to a standard LD cycle (Figure S4). Similar results were obtained when we repeated our analyses after controlling for body weight and behavioral activity levels. Lastly, consistent with age-related osteoporosis, the serum levels of

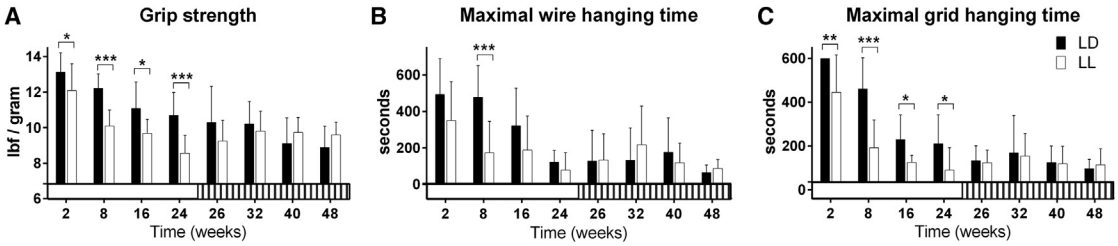

Figure 3. Exposure to LL Causes a Reversible Decline in Muscle Function Compared to Control Mice

(A–C) Forelimb grip strength, maximum wire hanging time, and maximum grid hanging time in both the LL and control (LD) groups (mean ± SD; n = 8–10 mice per group). Note that separate cohorts of mice were used at every time point. The data were analyzed using a two-way ANOVA followed by post hoc LSD. *p < 0.05, **p < 0.01, ***p < 0.001. See also Figure S3.

calcium, phosphate, and creatinine did not differ significantly between the LL and control groups (Figure S4).

Exposure to LL Alters the Immune System

The total white blood cell (WBC) count was relatively unchanged throughout the experimental phase and did not differ significantly between groups (Figure 5A). In contrast, both hemoglobin levels and hematocrit values were lower in the LL mice, particularly after 8 weeks of LL exposure (Figures 5B and 5C). Also at 8 weeks, the relative number of neutrophils was nearly twice as high in the LL group, and the LL mice had fewer lymphocytes (Figures 5D and 5E). The fraction of monocytes was higher in the mice in the LL group, particularly at 16 weeks (Figure 5F). In the recovery phase, the level of monocytes returned to control levels; however, the hemoglobin and hematocrit values did not return fully to control levels. With respect to the absolute number of leucocyte subtypes, we also measured significantly more neutrophils and significantly fewer lymphocytes in the LL group at 8 weeks; in contrast, absolute number of monocytes did not differ significantly between the two groups.

After 2 weeks into the experimental phase, the mice in the LL group had lower plasma levels of IL-1β and IL-10, and they had higher plasma levels of TNF-α compared to controls (Figure 5G). Total cytokine secretion measured as the area under the curve (AUC) also differed for IL-1β and TNF-α (Figure 5G; Table 1). After 8 weeks, lipopolysaccharide (LPS)-induced secretion of the pro-inflammatory cytokines IL-1β, IL-6, and TNF-α was higher in the LL group, whereas secretion of the anti-inflammatory cytokine IL-10 was lower in the LL group. At 24 weeks, LPS-induced cytokine secretion no longer differed between the two groups. Taken together, these data suggest that LL exposure induces a transient pro-inflammatory state.

DISCUSSION

Exposing *C57BL/6J* mice to LL for 24 weeks had significant effects on the in vivo activity of neurons in the SCN. In addition, LL caused a significant decrease in skeletal muscle function and caused microstructural bone changes characteristic of the early stages of osteoporosis. Finally, mice exposed to LL displayed transient changes in the immune system, including a pro-inflammatory state that resolved after prolonged exposure to light. Upon re-exposing the mice to an LD cycle, circadian period length, SCN neuronal rhythms, and immune function returned to pre-LL levels, while for the other parameters, the significant difference between the experimental and control group disappeared. Taken together, these results suggest that removing cyclic environmental cues causes a reversible state of physiological vulnerability.

Exposure to LL Decreases the Strength of Circadian Rhythms

Retinal photoreceptors receive light and project light-encoded information to the SCN via melanopsin-containing retinal ganglion cells and chemical photoreceptors [25, 26]. Continuous exposure to light causes desynchronization of SCN neurons [22], and in peripheral bodily tissues [27] in contrast to constant darkness [20]. The long-term effects of LL—as well as the system's capacity to restore rhythmicity after returning to a standard LD cycle—had not been studied previously and are highly relevant, given that circadian rhythms can be disrupted for extended periods, for example in shift workers or elderly persons. We confirm that short-term exposure to LL decreases neuronal amplitude [20] and demonstrate that long-term LL exposure further attenuates rhythmicity by approximately two-thirds throughout the 24 weeks of exposure. The strength of the SCN's rhythm of a given animal corresponded temporally with the strength of that animal's behavioral activity rhythm, suggesting that fluctuations in rhythm strength have a direct impact on overt behavioral rhythms.

LL Exposure Disrupts Skeletal Muscle Function

All mice had a decline in muscle function over time; this progressive decline in muscle function is likely due to the effects of aging [28]. The mice in the LL group had less grip strength than age-matched control mice, indicating that LL reduces muscle strength in addition to age. The mice in the LL group also had reduced physical endurance, reflected by decreased performance on the hanging tests, which require the mouse to maintain sustained force against gravity. Mice use all four limbs for hanging on a grid; in contrast, hanging on a wire is more complex and requires the coordination of several muscle groups, as well as dexterity and axial muscle strength. Even after 2 weeks in LL, the mice in the LL group showed a significant deficit, and this deficit remained throughout the experimental phase. Importantly, this decreased muscle function was independent of body weight or behavioral activity, indicating that the decrease in muscle function was not merely a reflection of weight gain.

A video analysis of mouse behavior prior to and after functional testing revealed that the two groups of mice were fatigued to a

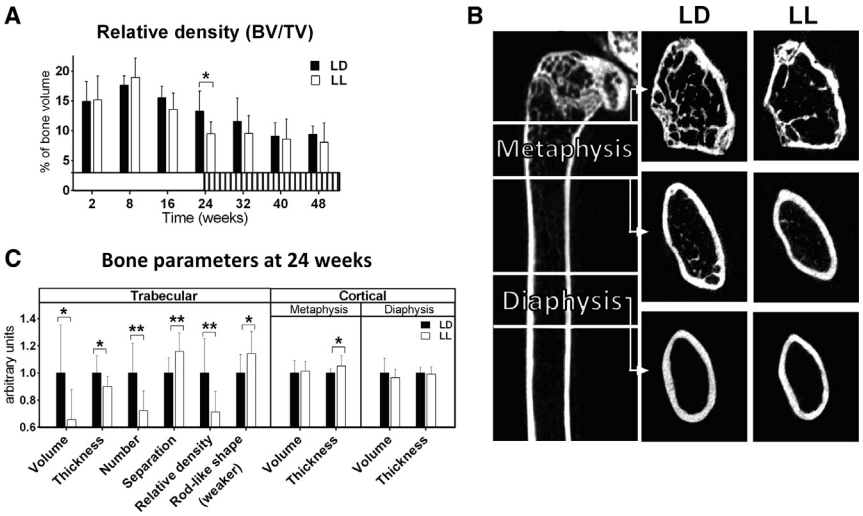

Figure 4. Continuous Exposure to Light Induces Clinical Features that Are Characteristic of Early Osteoporosis

(A) Relative trabecular density (BV/TV, measured as the ratio of bone volume to total volume) was measured in the LL and control groups at the indicated time points (mean ± SD; n = 8–9 mice per group). Note that separate cohorts of mice were used at every time point.

(B) Trabecular and cortical bone parameters measured at the end of the experimental phase (i.e., after 24 weeks) in LL and control mice (mean ± SD; n = 8–9 per group). All six trabecular bone parameters were significantly worse in the LL group.

(C) Examples of cross-sectional microCT views of the proximal and distal metaphysis and diaphysis in LL and control mice at 24 weeks.

The data were analyzed using a two-way ANOVA followed by post hoc LSD. *$p < 0.05$, **$p < 0.01$. See also Figure S4.

similar extent by the testing regime. They displayed similar levels of anxiety-related behavior, suggesting that the LL-induced decline in muscle function was not due to differences in motivation. We currently have no evidence to suggest that the decreased muscle function in the LL group was due to structural differences in muscle fibrosis, muscle damage (e.g., CK levels), macrophage levels in the muscle, muscle fiber type, or differences in mitochondrial function or muscle regeneration. Previous studies have linked circadian rhythm with muscle function by examining mice with mutations in their clock genes; specifically, these mice develop structural muscle changes [1, 29, 30] and diminished muscle function [1, 31]. However, because clock gene mutants may have impaired muscle function due to a direct effect of the clock gene mutation on the cell cycle [32], these models do not necessarily simulate disruptions in environmental rhythmicity. Our analysis did not reveal significant changes in muscle markers for immunological, degenerative or regenerative changes, energy regulation, or type of fiber. We cannot exclude that other markers would reveal differences but expect that pre- or post-synaptic signaling at the neuromuscular synapse is involved.

Continuous Exposure to Light Induces Clinical Features Reminiscent of Early Osteoporosis

Short-term (i.e., up to 8 weeks) exposure to LL had no effect on bone microstructure [33]. Indeed, the maturation of bone in both animal groups was similar to bone maturation in humans [33, 34]. Long-term exposure to LL had a negative effect on the microstructure of cancellous (i.e., trabecular) bone; after 24 weeks, the mice in the LL group had fewer trabeculae. The remaining trabeculae were more rod-like in shape, thinner, less voluminous, and more separated, thereby resulting in decreased BV/TV compared to control mice. These changes in trabecular bone are particularly striking, as C57BL/6 mice have a relatively low BV/TV compared to other mouse strains [33]. The progressive loss of trabecular bone is believed to play the most important role in the decline in bone strength associated with age-related osteoporosis [35]. The increased thickness of cortical bone in mice exposed to LL may also be an accelerated effect of aging, as cortical bone mass increases with age due to periosteal bone formation [35]; alternatively, it may represent a compensatory mechanism designed to maintain overall bone strength. Given its relatively large surface area, trabecular bone undergoes considerable turnover and remodeling; thus, osteoporotic changes are usually observed in trabecular bone first. Therefore, we feel that the changes in trabecular bone in mice exposed to LL are characteristic of early osteoporosis. In previous studies, light at night has been associated with lower rather than higher corticosterone levels [20, 36]. Since increases, not decreases, in corticosterone have been associated with muscle wasting and osteoporosis, we do not expect that changes in levels of corticosterone were a direct trigger for the effects we observed. For a mechanistic explanation for the observed differences, future studies should include measurement of markers of bone turnover, such as P1NP and CTX.

Of note, the levels of both calcium and phosphate were unaffected in the mice in the LL group, consistent with age-related osteoporosis in humans [2]. Moreover, general kidney function, which was assessed by measuring creatinine levels, was also unaffected by LL exposure.

In human studies, female shift workers have an increased risk of bone fractures [12] and decreased bone mineral density [11]. These observational studies cannot be used to determine causality; thus, our results indicate for the first time that disrupted environmental rhythms are a causal factor in the decline in bone microstructure. Per and Cry mutant mice display increased bone mass and osteoblast activity [37], while Bmal1 knockouts display ectopic calcification and abnormal endochondral ossification [3, 37, 38].

Circadian Disruption and the Immune System

Two weeks of LL exposure mildly affected LPS-induced cytokine secretion. Eight weeks of LL exposure induced a higher number of neutrophils, pro-inflammatory cells in the innate immune system; thus, LL appears to induce a heightened pro-inflammatory state. Consistent with this notion, the mice in the LL group had higher LPS-induced secretion of the pro-inflammatory cytokines IL-1β, IL-6, and TNF-α, coupled with decreased secretion of the anti-inflammatory cytokine IL-10. These differences were no longer observed after 24 weeks of LL exposure, suggesting

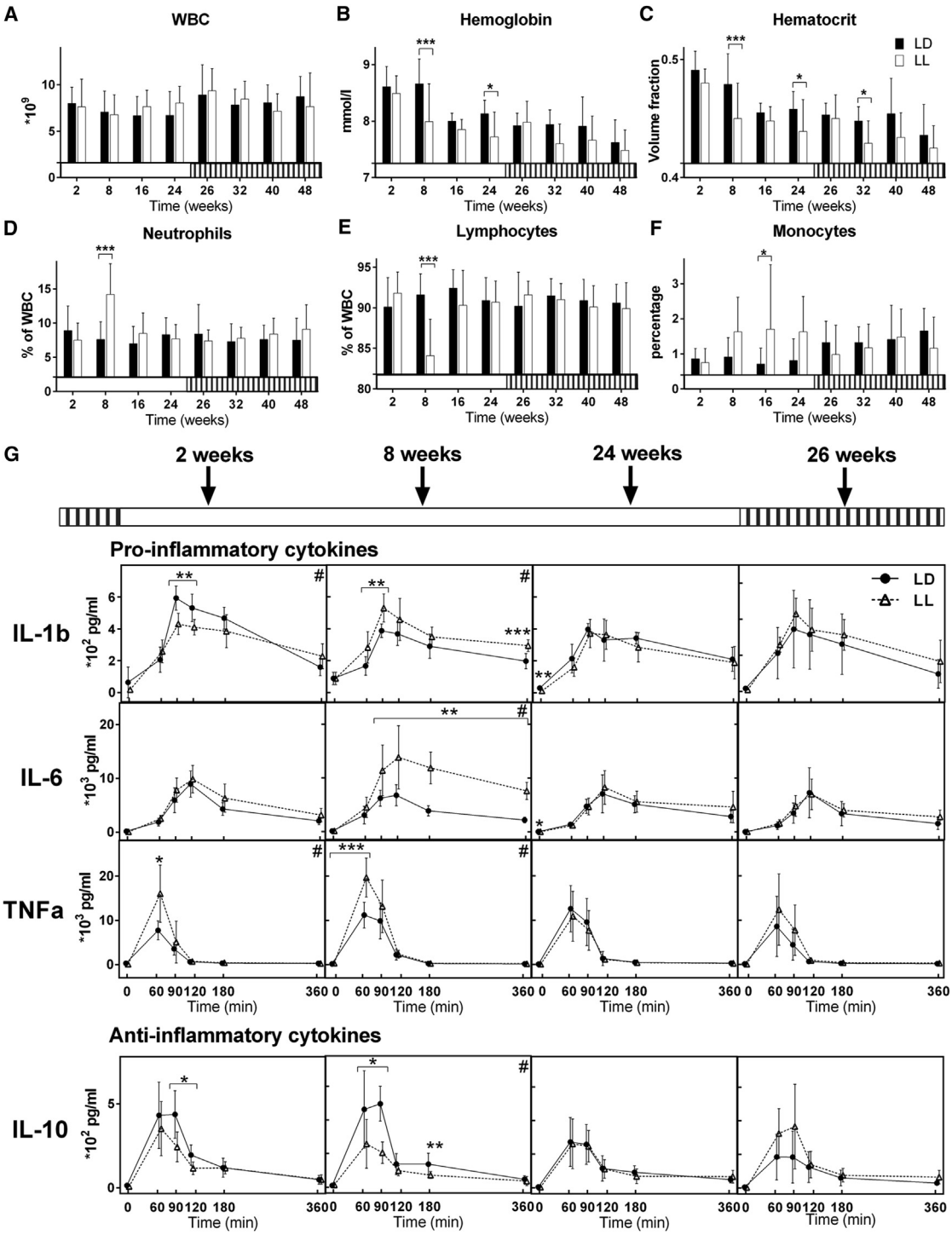

Figure 5. Continuous Exposure to Light Reversibly Alters the Homeostatic and Responsive States of the Immune System

(A–F) The indicated values were measured in LL and control mice at the indicated times; WBC, white blood cell count (mean ± SD; n = 8–10 mice per group). Note that separate cohorts of mice were used at every time point.

(G) Plasma levels of IL-1β, IL-6, TNF-α, and IL-10 were measured prior to (time 0) and 60, 90, 120, 180, and 360 min after an injection of low-dose LPS at the indicated experimental time points (mean ± SD; n = 5–12 per group).

The data were analyzed using a two-way (A–F) or repeated-measures (G) ANOVA, followed by post hoc LSD. *p < 0.05, **p < 0.01, ***p < 0.001. # in the top-right corner in (G) indicates that the AUC differed significantly between the two groups.

Table 1. Altered Total Cytokine Production in Response to LPS Challenge in LL

	2 Weeks		8 Weeks		24 Weeks		26 Weeks	
	LD	LL	LD	LL	LD	LL	LD	LL
Pro-inflammatory Cytokines								
IL-1β (× 10^4)	6.7 ± 0.9	5.0 ± 1.0**	4.7 ± 1.0	6.2 ± 1.6**	4.8 ± 0.7	4.4 ± 1.0	3.4 ± 2.6	5.0 ± 1.1
IL-6 (× 10^5)	8.0 ± 2.3	8.5 ± 2.8	7.5 ± 1.8	13.8 ± 5.6**	6.4 ± 2.2	7.3 ± 2.3	4.7 ± 3.6	6.5 ± 2.3
TNF-α (× 10^5)	4.7 ± 1.7	9.2 ± 4.1*	9.1 ± 3.5	14.1 ± 3.7**	9.5 ± 4.4	7.9 ± 4.1	4.5 ± 4.4	8.5 ± 5.6
Anti-inflammatory Cytokine								
IL-10 (× 10^4)	4.4 ± 1.4	5.8 ± 9.2	4.6 ± 1.8	2.5 ± 0.7**	2.9 ± 1.2	2.6 ± 1.2	1.7 ± 1.5	3.4 ± 1.7

The AUC of each cytokine is shown in pg/ml/min. Note that separate cohorts of mice were used at every time point. Data are represented as mean ± SD (n = 5–12 per group). Two-way ANOVA, followed by post-hoc LSD. Asterisks indicate difference between control and LL groups: *$p < 0.05$ and **$p < 0.01$.

that the effect of LL on the immune system shows desensitization and/or that compensatory mechanisms may have been activated. Identifying such compensatory mechanisms would provide valuable information.

Previous studies have linked disruptions in environmental rhythms with impaired immune function. For example, shift workers have an increased risk of cancer [9] and metabolic syndrome [39], both of which are related to immune system dysfunction [40]. Shift workers do not have altered baseline cytokine levels [41], and their immune response to challenges has not been investigated. Previous animal studies have suggested that circadian disruption is a causal factor in altered immune system function. For example, mice subjected to a chronic (i.e., 4-week) jet lag protocol have an enhanced response to LPS challenges [4], and intestinal irritant-induced colitis is more aggressive in mice that are chronically phase shifted [42]. Moreover, exposing rats to LL increases mortality following LPS-induced sepsis [43]. In our experiments, we used a low, non-lethal dose of LPS; therefore, we were able to quantify the effects of an immune stimulus that more closely resembles the inflammatory response in human sepsis [44].

Recovery of Health versus Stability of Health upon Returning to a Normal Environmental Cycle

After returning to a standard LD cycle (i.e., the recovery phase), the mice in the LL group no longer had impaired muscle performance or deficits in trabecular bone microstructure. It is difficult to assess whether restoring the LD cycle leads to a bona fide recovery of health, as bone microstructure and muscle function naturally decline with age. Nevertheless, in the recovery phase, many health parameters either stabilized or improved slightly, and none of the parameters measured continued to decline, while muscle function and bone microstructure have the potential to decline to much lower levels, as observed in very old mice or models of severe disease [33, 45].

Immune parameters recovered to values before LL treatment and in the SCN, neuronal rhythmicity recovered instantaneously after returning the mice to a standard LD cycle. Importantly, this rapid recovery led to a large amplitude rhythm that was properly phased. The trough upon the first exposure to darkness was particularly large, suggesting that this sudden, first absence of light input acts as a ''phase-resetting'' stimulus for the majority of SCN neurons. Our results are consistent with a previous report in which the SCN's rhythm recovered almost immediately following a short bout of LL exposure [46]. Because neuronal activity is the first step in generating the output signal and because this activity drives the release of both neurotransmitters and humoral signals, restoring the SCN's output signal and thereby boosting the rhythm in the sympathetic outflow [47] will have immediate consequences for all peripheral systems that are under control of the autonomic nervous system.

Clinical Relevance

Exposing animals to LL is an important model for intensive care settings and nursing homes, in which lighting can fluctuate so little throughout the 24-hr period that patients usually fail to entrain to these cycles [48–50]. For example, rhythms in behavior, body temperature, corticosteroid levels, heart rate, and melatonin levels are often disrupted—or even abolished—in intensive care patients [51–54]. Ironically, exposure to a robust environmental cycle may be particularly relevant to severely ill patients, as these patients could benefit considerably from a robust immune response. Studies have shown that preterm infants in neonatal intensive care units have improved sleep patterns and gain weight faster when exposed to a robust LD cycle [55–58]. In addition, nursing home residents have improved sleep and higher levels of physical activity [59].

In the present study, we used a condition of LL as a paradigm to induce rhythm disturbances, rather than light-dim light or a chronically shifting LD schedule, since LL has displayed consistent and strong effects and it is most comparable to certain artificial light settings, for example, the intensive care setting. Given our present results, it is of importance to perform studies with paradigms that mimic light pollution in modern society in other ways and to explore their effect on bone, muscle, and immune function.

As 18% of elderly adults have decreased muscle strength [60], 6%–21% have osteoporosis [61], and immune system dysregulation can aggravate age-related pathologies [62], large segments of the elderly population are at increased risk for frailty. While intuitively health is associated more with the immune system, it is generally accepted (e.g., in the clinic) that bone and especially muscle function is a strong indicator for general health, correlating highly with life expectation. A frail state in the elderly could be explained by a decline in their circadian system, since the changes in rhythm amplitude in the SCN in the LL group are reminiscent of rhythm changes that occur in the clock of aged individuals [63]. Therefore, the LL-induced decline in mice may represent the contribution of an ''aged'' clock to the age-related decline in health.

Conclusions

Here, we provide insight into the long-term effects of disrupted environmental rhythmicity on several major health parameters, and we provide evidence that the majority of these effects are reversible. We conclude that complex temporal relationships involved in daily fluctuations in muscle function, bone microstructure, and immune function are disrupted by exposure to LL, and this disruption underlies the observed changes in health. The effect of LL-induced disruption of the SCN and consequent effects for SCN output are expected to be intrinsically related with secondary effects such as sleep disturbances, changes in the hypothalamic–pituitary–adrenal axis and autonomic nervous system, etc. The contribution of their effect to the observed decline in health-related parameters cannot be disentangled from the immediate circadian disorders induced by LL. Yet, the important message is that the environmental LL condition is sufficient to trigger a cascade of effects, leading to frailty.

These results create new opportunities for prevention and treatment programs, particularly for frail individuals, such as intensive care patients, nursing home residents, and the elderly. Our results are also highly relevant to large segments of the population, as three-quarters of the world's population is routinely exposed to artificial light during the night [6]. We propose that long-term prospective studies should be performed to examine the health effects of increasing diurnal light levels in such settings. For example, in addition to increasing light levels during the day [64], light exposure during the night can be reduced easily without compromising patient safety [65]. The long-term effects of a robust LD cycle on muscle function, bone microstructure, and the immune system are currently unknown in humans. Our study provides compelling evidence that the detrimental effects of chronic LL exposure warrant further investigation.

EXPERIMENTAL PROCEDURES

All experiments were approved by the Leiden University Medical Center's Ethics Committee for Animal Experimentation. A detailed description of reagents and protocols including study design, behavioral and electrophysiology data collection and analysis, muscle function tests, tissue processing and analysis, and statistics can be found in Supplemental Experimental Procedures.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures and four figures and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2016.05.038.

AUTHOR CONTRIBUTIONS

E.A.L., C.P.C., J.H.M., J.W.A.S., A.M.A.-R., H.H.S., B.G., and C.W.G.M.L. designed the experiments. E.A.L., C.P.C., M.v.P., S.R.d.K., R.P.M.S., J.H.L.T.v.G., S.L.V., M.v.d.V., and K.E.d.R. performed the experiments. E.A.L. and J.H.M. wrote the paper, and C.P.C., A.A.R., M.v.P., S.R.d.K., S.L.V., H.H.S., B.G., J.S., K.E.d.R., and C.W.G.M.L. provided comments on the final manuscript.

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