Disruption of circadian rhythms and delirium, sleep impairment and sepsis in critically ill patients. Potential therapeutic implications for increased light-dark contrast and melatonin therapy in an ICU environment.

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Abstract

The confinement of critically ill patients in intensive care units (ICU) imposes environmental constancy throughout both day and night (continuous light, noise, caring activities medications, etc.), which has a negative impact on human health by inducing a new syndrome known as circadian misalignment, circadian disruption or chronodisruption (CD). This syndrome contributes to poor sleep quality and delirium, and may impair septic states frequently observed in critically ill patients. However, and although the bidirectional crosstalk between CD with sleep impairment, delirium and inflammation in animal models has been known for years and has been suspected in ICU patients, few changes have been introduced in the environment and management of ICU patients to improve their circadian rhythmicity. Delirium, the most serious condition because it has a severe effect on prognosis and increases mortality, as well as sleep impairment and sepsis, all three of them linked to disorganization of the circadian system in critically ill patients, will be revised considering the functional organization of the circadian system, the main input and output signals that synchronize the clock, including a brief description of the molecular circadian clock machinery, the non-visual effects of light, and the ICU light environment. Finally, the potential usefulness of increased light/dark contrast and melatonin treatment in this context will be analyzed, including some practical countermeasures to minimize circadian disruption and improve circadian system chronoenhancement, helping to make these units optimal healing environments for patients.

Keywords: chronodisruption, circadian light, melatonin, intensive care unit, circadian rhythms, chronoenhancement.
Introduction

The confinement of critically ill patients in an artificial bubble with a constant environment is a common characteristic of intensive care units (ICU). The acclimatization of ICUs allows for maintaining a constant temperature during 24 h. Artificial light illuminates both days and nights with the same intensity and spectrum. Continuous noise, patient care interactions, the use of artificial ventilation and medications, all conducted 24 hours a day, contribute to the disruption of the circadian system or chronodisruption (CD), and to the poor sleep and paucity of time spent in restorative sleep stages observed in critically ill patients (CIP). However, in spite of the fact that the sleep impairment of ICU patients has been known for years, few changes have been introduced in the environment and the management of ICU patients to improve their circadian rhythmicity.

The resistance to change in clinical practice may be largely due to the dominant concepts, derived from the prevailing homeostatic paradigm, impinging on all aspects of physiology and medicine. In fact, constancy is not only restricted to the ICU environment, it also affects all of our physiological paradigms and medical therapies.

According to the classic concept of homeostasis, healthy systems are self-regulated to reduce variability and maintain physiological constancy. Thus, it is thought that our body responds to external/internal challenges through negative feedback loops in such a way that each perturbation is corrected to a physiological reference value, consequently ensuring constancy in our internal milieu. In reality, biological systems do not behave in such a homeostatic way. Admittedly, various near-homeostatic mechanisms do exist, e.g., those controlling the temperature, levels of glucose or calcium in the circulation. However, a close look at frequently taken time series of these parameters reveals systematic fluctuations due to influences of the endogenous, circadian oscillator system [1, 2, 3]. Even the existence of a feedback loop does not necessarily lead to homeostatic behavior. A classic example is that of glucocorticoids, which display a circadian rhythm with an amplitude that is among the highest known in chronobiology [4, 5], despite a feedback to the upper levels of the respective hormonal axis.

It is therefore a misconception to uncritically assume that homeostatic properties of a biological system are synonymous with constancy. A consequence of the homeostatic misconception is the widely accepted, yet very often unjustified belief that an adequate constant input value is better than a variable one, because constant inputs are assumed to reduce stress to the regulatory systems and help preserve health. This questionable conclusion leads to many practical implications. For example, an infusion at a constant rate is wrongly believed to provide constant plasma levels of the infused drug; a drug effect is thought to be equal irrespective of its administration time; and constant and stable environments are presumed to promote healing in intensive care unit treatments [6].

However, contrary to the classic concept of reactive homeostasis, the output of a wide variety of systems, such as the normal human heartbeat, body temperature, brain neurotransmitter levels and sleepiness, fluctuates periodically in a complex manner, even under constant environmental conditions. In addition, numerous chronobiological experiments show that, under constant physiological conditions, non-linear regulatory systems are operating far from the thermodynamic equilibrium and that maintaining constancy is not the purpose of physiologic control. These insights are not entirely new, but they indicate that it is high time for a paradigm change and further study on how environmental rhythmicity helps prevent circadian dysfunctions and maintain a healthy state in CIP.

Therefore, quite the opposite to the currently widespread, yet obsolete opinion, constancy has a negative impact on human health by inducing a new syndrome, which is now being called by different names, such as circadian misalignment, circadian disruption or chronodisruption (CD). Loss of contrast between day and night conditions, and particularly in terms of light-dark alternation, is one of the most frequent causes of CD in our ICUs. Chronodisruption could be defined as a relevant impairment of the
internal temporal order among different physiological, biochemical and behavioral variables, and/or between them and the environment. In recent years, the impact of CD on human health has become an important concern. An increasingly expanding body of experimental evidence links CD with the increased risk of developing various diseases, and the aggravation of pre-existing pathologies, such as premature aging, cancer, cardiovascular diseases, cognitive impairment and mood disorders. Obesity and metabolic syndrome are two additional pathologies that are closely associated with CD in the general population.

The aim of this review is to summarize the literature on the potential therapeutic implication of increasing light-dark contrast for preventing circadian disturbances and promoting the healing of CIP by using light during the day and potentiating melatonin levels during the night. The use of personalized circadian markers such as DLMO and the human phase-response curve is also discussed.

**Functional organization of the human circadian system**

The sleep-wake rhythm and the remaining circadian rhythms of the human body are generated, coordinated, and synchronized both internally and to external environmental cues, by the circadian system (Figure 1). This system is composed of several structures, including a central pacemaker, peripheral oscillators, molecular clock machinery, the eyes, the pineal gland and neural and humoral input and output pathways.

The major circadian pacemaker is the suprachiasmatic nucleus (SCN), located in the anterior hypothalamus [7, 8]. The electrical activity of an individual neuron from the SCN can oscillate with a period of approximately 24 hours in response to the operation of a molecular clock that comprises several positive and negative feedback loops, consisting of clock genes and their corresponding proteins. The single rhythm output that sends a temporal signal to the rest of the body is generated by the SCN as the result of the activity of a multi-oscillator network of neurons synchronized by cell-to-cell coupling [9].

In addition to the central pacemaker, most - if not all - tissues and organs possess their own circadian oscillators with a specific phase. Under physiological conditions, these are orchestrated by the SCN through physical (temperature), neural (selective sympathetic and parasympathetic projections) and humoral (glucocorticoids and melatonin) signals. The maintenance of an adequate temporal order of the rhythms generated by different circadian oscillators is a necessary condition to preserve a healthy state and promote healing.

However, under certain conditions, peripheral oscillators behave as autonomous oscillators, generating their own circadian structure. This characteristic provides a good argument against a simple hierarchical master-slave organization, and basically supports more a resonant network. Peripheral clocks will permit individual tissues and organs to respond differently to generic signals generated by the SCN [10] and to react selectively to certain environmental cues, such as feeding time. But let’s summarize the main signals that wind the clock, its main output signals and the molecular clock machinery.
Figure 1. General organization of the mammalian circadian system under natural and ICU conditions. **Input signals:** cyclic environmental cues (*zeitgebers*) can synchronize the activity of the central pacemaker and peripheral oscillators. **Circadian clocks:** the oscillatory machinery is composed of a central pacemaker and peripheral oscillators. The suprachiasmatic nuclei (SCN) is considered to be the major pacemaker of the circadian system, driving circadian rhythmicity in other brain areas and peripheral tissues by sending them neural (Autonomic nervous system, ANS), humoral (melatonin and cortisol rhythms) and physical (core body temperature rhythm) signals. Most peripheral tissues and organs contain circadian oscillators. Usually, they are under the control of the SCN. **Outputs:** central pacemakers and peripheral oscillators are responsible for the daily rhythmicity observed in most physiological and behavioral functions. Some of these overt-rhythms (physical exercise, core body temperature, sleep-wake cycle and feeding time), in turn, provide feedback, which can modify the function of SCN and peripheral oscillators (*zeitnehmers*). Under natural environmental conditions, biological and behavioral variables display robust and internally synchronized rhythms. However, in the ICU environment, characterized by low or absent contrast between day and night, most *zeitgebers* are lost. This leads to circadian disruption, a risk factor for the development of serious pathological consequences.

**Circadian inputs: zeitgebers**

The action of the SCN is sufficient for circadian rhythms to appear. However, if these rhythms are synchronized to environmental cycles, the circadian pacemaker has to be periodically set by the action of certain environmental factors (synchronizers or *zeitgebers*), which oscillate rhythmically and which, taken as a whole, act as the clock input pathway. Among these *zeitgebers*, the most important is the light-dark cycle, although meal times, scheduled exercise, sleep and social contacts also act as synchronizers [11, 12, 13].

Photic information is conveyed to the SCN through the retinohypothalamic tract, which is formed by axons from intrinsically photosensitive retinal ganglion cells (ipRGCs), a subpopulation of RGCS that do not take part in conscious image formation. These cells contain a photopigment, melanopsin (maximal sensitivity to blue light from 460-480 n), which is not present in rods and cones [14]. The presence of
these SCN-projecting photosensitive cells explains why the circadian rhythms of blind rats lacking cones and rods remain synchronized to the environmental light-dark cycle [15, 16]. For a more extensive review, see Bonmati-Carrion et al. [17].

Circadian overt-rhythms. Melatonin

The SCN uses neural projections, humoral mediators, such as melatonin and cortisol, and physical signals, including the central temperature rhythm, to transmit its temporal information to other brain structures (that participate, for example, in sleep-wake rhythm regulation), neuroendocrine centers and peripheral organs.

One of the best characterized humoral mediators of the SCN is melatonin. This hormone is involved in the regulation of sleep, and both circadian and seasonal rhythms [18, 19]. Its synthesis is subjected to double regulation, because it responds to noradrenergic stimulation activated during the subjective night of the SCN, and at the same time it is directly inhibited by light [20].

The production of this hormone shows a marked circadian rhythm, with low plasma levels during the day and a large peak at night, regardless of the nocturnal or diurnal characteristics of the organism [21]. Thus, melatonin is also known as the “chemical expression of darkness” [22]. The great stability of the melatonin cycle and the fact that it is produced during darkness means that it can be used by organisms as a daily clock, informing them of the arrival of night, and as a calendar that tells them which season they are in [23, 24].

Melatonin exerts numerous physiological functions and displays pleiotropic effects throughout the body. It is involved in the regulation of circadian rhythms by modulating the SCN electrical activity, and by phase-shifting the circadian clock. These chronobiological effects of melatonin are mediated by its interactions with the MT₁/MT₂ membrane receptors [25]. While MT₁ activation modulates the SCN electrical activity, MT₂ seems to be involved in the phase-shifting effects of melatonin, which are dependent on circadian time, duration of exposure to melatonin and melatonin receptor sensitivity. Thus, when exogenous melatonin is administered late in the subjective day (dusk) or in the early phase of the night, it phase-advances the circadian clock; by contrast, delays in the circadian rhythms or no response are observed when melatonin is administered late in the subjective night or early in the day [26].

In addition to its role as a chronobiotic agent [27], melatonin has antitumoral, neuroprotective, immunomodulatory, anti-inflammatory and antioxidant properties [25, 28]. The protective effects of melatonin against oxidative stress may partially involve its interactions with the enzyme quinone reductase 2 and RORα receptors, as well as its capacity to act as a direct scavenger of free radicals [29].

The activity of the SCN in generating circadian rhythms and its ability to respond to some of these rhythms are inseparable. For example, the sleep-wake rhythm can be rightly considered as an output of the SCN, but, in turn, this rhythm indirectly determines the exposure to light, feeding time and physical exercise, all of which are considered to be zeitgebers of the circadian system. In Roenneberg's terminology [30, 31], those feedbacks that are both inputs and outputs of the circadian system are referred to as zeitnehmers (from the German “time taker”). In addition to the sleep-wake rhythm, melatonin and physical exercise can be considered zeitnehmers.

The circadian molecular clock

Both in the SCN and the peripheral oscillators, each cell behaves as an autonomous circadian oscillator. At the cellular level, circadian oscillators result from the existence of positive and negative feedback
loops in which the products of the expression of some genes inhibit their own transcription, generating a rhythmicity of around 24 hours [32]. The main components that have been identified in the clock of mammals are: Clock and Bmal1 genes as positive elements, and Per genes (Per1, Per2 and Per3) and the cryochromes (Cry1 and Cry2) as negative elements [33].

The positive arm of the feedback loop is composed of CLOCK and BMAL1 proteins, which heterodimerize in the cytoplasm and translocate to the nucleus, where they activate the transcription of certain target genes (Per, Cry, Rev-Erbα) and clock controlled genes (CCGs, including key regulators of the cell cycle and metabolism). The CCG can make up to 10% of the genome in some tissues.

The negative feedback loop comprises the heterodimers PER:CRY, which translocate to the nucleus where they suppress their own transcription by inhibiting CLOCK and BMAL1 activities. Meanwhile, the protein REV-ERBa suppresses Bmal1 transcription by binding to the elements involved in the response to Rev-erbaROR, which are present in the Bmal1 promoter. Consequently, the RNA levels of Bmal1 diminish, while those of Per and Cry increase. When the PER:CRY heterodimers suppress their own transcription at the nuclear level (by acting on CLOCK-BMAL1), they also inhibit the transcription of Rev-erba, permitting the transcription of Bmal1 to be activated.

The approximately 24 hour rhythmicity of the molecular clock mainly derives from post-translational modifications such as phosphorylation and ubiquitination, processes which affect the stability and translocation of the clock genes to the nucleus [34]. In this way, the casein kinase 1 epsilon (CK1ε) and casein kinase 1 delta (CK1δ) are critical factors that modulate the functioning of the clock. The importance of these post-translational modifications of the clock components is borne out by studies showing that mutations in CK1ε have an effect on circadian periodicity [35]. CK1ε phosphorylates PER proteins, so that they are not immediately available to form dimers, leading to a circadian cycle with a longer period. When this gene is mutated and the resulting protein has reduced phosphorylation activity, PER proteins are internalized into the nucleus more rapidly, shortening the cycle.

**Chronodisruption-induced pathologies in the ICU context: Delirium, sleep impairment and sepsis.**

**Delirium**

The environment in a critical care unit is one in which patient circadian rhythms are continuously disturbed. The characteristics and pathologies of the patients usually require treatments that predispose them to sleep and circadian disruption [36]. CD is associated with the frequent appearance of different complications, including delirium, which influences the vital and functional prognosis in critically ill patients [37]. In fact, delirium leads to a worse prognosis, with increased mortality, ventilation time and hospitalization [37, 38, 39], mainly in the elderly [40], as well as the possible future development of dementia [38, 41]. Therefore, modifying the factors that can improve circadian rhythms in ICU patients would seem to be a smart strategy.

Delirium is a neuropsychiatric syndrome characterized by an altered mental status, acute fluctuations in the level of attention, cognitive impairment and flattening of the sleep-wake rhythm. It has a prevalence of approximately 10-31% in hospitalized patients [42, 43], which increases to 50% in the ICU [39, 43], and can even reach as much as 80% depending on the characteristics of the patients studied [38]. Although the true pathogenesis of delirium is not fully understood [45], it is known to be caused by neuronal dysfunction secondary to systemic disorders, influenced by the coexistence of multiple risk factors and predisposing factors [39, 46, 47], such as the severity of disease, prior cognitive impairment, measures of restraint, medication (benzodiazepines and antipsychotics), mechanical ventilation, visual and hearing impairment and dehydration. Isolation, which implies no visits, the absence of daytime sunlight exposure and physical restraint have also been considered a risk factor for the development of delirium [48].
Evidence that links the alteration of the circadian system to delirium has also been reported. The first line of evidence is based on the daily fluctuation in the symptoms of delirium, similar to the periods of sundowning shown by subjects with Alzheimer's disease, in which the disruption of circadian rhythms correlates with the severity of sundowning [49]. Thus, some authors suggest that sundowning in dementia and the agitation of delirium possess a common pathway that could involve circadian disruption [50]. The second line derives from the impairment of a circadian zeitnehmer, the sleep-wake pattern, which also shows disturbances in patients with delirium [46, 51, 52]. Sleep loss, both acute and chronic, is known to cause cognitive impairment [53, 54, 55], and ICU patients usually complain of insomnia, poor quality of sleep and sleep fragmentation [36,56]. In fact, sleep deprivation in the ICU was reported to be a risk factor for the development of delirium over the following days [57]. Moreover, the presence of prior delirium was also an independent factor associated with the onset of REM reduction [51].

In addition, the circadian rhythm of melatonin, the hormone that transmits the clock information of darkness, appears to be disrupted in critically ill patients [58, 59, 60, 61] with either sepsis [59, 62], or mechanical ventilation and intravenous sedation [60], although other authors [59] report that the circadian rhythm was preserved in nonseptic ICU patients, and only patients with sepsis displayed no melatonin rhythm. However, it must be considered that the eyes of sedated patients remain closed most of the time. The absence of effective photic zeitgebers in these cases could cause melatonin production to retain a circadian rhythm, albeit phase delayed or with a free running rhythm [63, 64].

**Sleep impairment**

Critically ill patients suffer from altered sleep, which is usually severely fragmented, distributed between day and night, with an increased number of arousals and awakenings, decreased time in slow wave and REM sleep stages and increased time in stage 1 and 2 [36, 61, 65, 66, 67], while the total sleep time over 24 hours may be either normal or diminished [65, 67]. The main elements involved in this disturbance are noise, nocturnal light, low levels of diurnal light, pain or illness and consequent psychosocial stress, patient-care activities, mechanical ventilation and medications [66, 67, 68, 69, 70, 71, 72]. In the case of mechanical ventilation, contradictory results have been found, which show secondary disturbances of sleep [63, 64] and melatonin secretion patterns [73] or an absence of impact of mechanical ventilation in sleep [74].

Although one study was not able to show any differences in circadian rhythms between patients with or without a confusional state in the ICU [75], there is a growing body of evidence that sleep disturbances are associated with adverse outcomes [76, 77, 78], and a relevant link between sleep disturbances and delirium has also been found [79, 80].

In fact, noise and patient care activities were identified by PSG recording as the most relevant factors associated with microarousals and awakenings [70], and the patients themselves identified light, general noise and the conversations of caregivers as the most important factors on a survey concerning the causes of sleep disturbance in the ICU [72]. Other etiological factors involved in the impairment of sleep include mechanical ventilation, the severity of disease and the sedative drugs used, such as benzodiazepines [81].

The influence of light and the absence of lighting contrast between day and night have been also proposed as a sleep disruptor. However, in most studies light at night was not addressed as an isolated factor, but rather in association with other activities, such as the patient care [81]. A study using a simulated ICU environment [82] provided evidence supporting the significance of light at night as a sleep disruptor. After monitoring various ICUs, nocturnal light and noise were recreated in the laboratory and the sleep of healthy volunteers was recorded by PSG. Light levels during the night produced an alteration in normal sleep architecture and melatonin rhythm, which improved after using a mask and earplugs. However, patients perceived light at night as a disruptive element of their sleep, but secondary to other factors, such as noise and phlebotomy [83], although the lack of an objective measurement of the real light exposure reduces the validity of this conclusion.
Sepsis

Immune cells are equipped with molecular clocks synchronized through the suprachiasmatic nucleus, which influences their rhythms through mechanisms that are not totally understood, such as the hypothalamus-pituitary-adrenal axis and the sympathetic nervous system [84]. The secretion of glucocorticoids, well known for their anti-inflammatory properties mediated through control over the production of cytokines and leukocyte trafficking properties, exhibits a circadian rhythm under SCN and adrenal clock control [84].

Inflammation mediators also exhibit a circadian rhythm associated with the molecular clock components, as demonstrated in knockout mice, where certain inflammatory cytokines, such as IL-6 and TNF-α are associated with the presence of BMAL1 or IFNγ in association with PER2 protein, which also regulates the rhythms of natural killer cells [85].

Evidence derived mainly from experiments in rodents has clearly demonstrated a link between circadian rhythms and sepsis. Animals subjected to chronic jet-lag or to other chronodisruptive conditions show increased proinflammatory responses [86, 87]. This has also been demonstrated to occur in models of sepsis in which wild-type animals are subjected to constant darkness or light, as compared to those subjected to regular LD cycles [88].

In humans, the connection between circadian disruption, sleep and inflammation is more complex, probably reflecting the bidirectional influence among them. Sleep architecture is influenced by the inflammatory states occurring in sepsis, since inflammatory cytokines IL-1 and TNF-α appear to promote deep NREM sleep [89]. A placebo-controlled study, in which different doses of endotoxin were administered at night to simulate a limited sepsis model, concluded that low doses producing an inflammatory response increased total NREM deep sleep, whereas higher doses impaired the general sleep architecture [90]. However, the hypothesis that sleep is involved in immunity in humans has not been entirely confirmed, since the same inflammatory response to endotoxin administration remained unchanged in patients with acute sleep deprivation, with respect to normal sleepers [91]. However, sleep and circadian rhythms do seem to exert some kind of influence on immunity. Circadian misalignment and sleep deprivation increase the risk of diabetes and metabolic syndrome, two pathologies sharing a common inflammatory base, by modifying the level of inflammatory mediators in previously healthy individuals [92, 93]. This evidence supports the hypothesis of a reciprocal influence between sleep and inflammation.

Melatonin, the major hormone of the circadian system, is also an important mediator of the inflammatory response. In mice with a deletion of MT1 and MT2 melatonin receptors, experimental sepsis generated a greater inflammatory response, mainly due to the anti-inflammatory and antioxidant role of melatonin [94]. In another model of sepsis, exogenous melatonin or ramelteon increased the survival of both double knockout rats for melatonin receptors and wild type rats. This effect disappeared with the administration of a melatonin receptor antagonist, such as luzindole [95]. Melatonin thus directly or indirectly appears to optimize the inflammatory response in sepsis. However, in humans, the evidence is less conclusive. Therefore, controlled studies focused on assessing the efficacy of exogenous melatonin in sepsis patients are necessary.

The association between circadian disruption on the one hand and delirium, sleep impairment, and to a lesser degree, sepsis on the other, is undeniable, but their causal links in humans remain unknown, and most likely will be multifactorial. The only potentially modifiable factor is the ICU environment, especially considering that its very low day/night contrast poses many risk factors for the development of delirium and sleep impairments. The little evidence available supporting the different current therapeutic options for treating delirium in critically ill patients requires that direct attention be paid to the possible preventive strategies [47], including a combination of measures targeting the entrainment of these patients circadian rhythms, as will be discussed later.
Non-visual effects of light. The phase-response curve for light.

As previously mentioned, the non-visual effects of light entail circadian photoreception through the ipRGCs by means of melanopsin. These ganglion cells are implicated in different non-visual functions related to circadian photoentrainment, negative masking, sleep regulation, melatonin secretion inhibition and the pupillary light reflex (Figure 2).

A negative masking occurs when locomotion is reduced by light in mice and other nocturnal mammals. In this case, it has been demonstrated that melanopsin is required for maximal and sustained response, while rods and cones would drive positive masking (increasing locomotion) at very low intensities, which could imply that the image-forming system helps guide locomotion [96, 97].

The pupillary light reflex (PLR) reduces rod and cone saturation by light. Driven by ipRGCs, it improves resolution by increasing the depth of field. These cells are needed for maximal and sustained pupil constriction, perhaps to compensate for light adaptation in rods and cones [98]. In mice lacking rods or cones, the PLR begins only in bright light, but it is driven until completion [99, 100], which is why this technique is used in humans to evaluate the integrity of this non-photic input to the SCN.

With regard to sleep regulation, in nocturnal rodents, a pulse of light during the dark period induces sleep and c-fos expression in the VLPO nucleus [101], a sleep-promoting brain area, while a pulse of darkness administered during the light period can induce awakening [101]. Melanopsin-null mice lack these
effects, and only show perturbations in sleep homeostasis [101]. These findings can be applied to diurnal organisms, but light would promote awakening, and darkness would facilitate sleep [102].

Suppression of pineal melatonin also occurs in rodless/coneless mice under high-intensity light [99], a process which requires melanopsin. Moreover, some visually impaired people also show this melatonin suppression, with a spectral sensitivity consistent with melanopsin signaling [103, 104], indicating the implication of ipRGCs in this process.

Circadian photoentrainment refers to the entrainment of an organism's circadian rhythm to the pattern of light and dark in the environment; it depends on rods and cones, but most importantly, on ipRGCs. Thus, it is known that some visually impaired subjects with no image vision due to rod/cone degeneration can still be photoentrained, since their ipRGCs remain unaffected [105]. However, studies with melanopsin-null mice that presented phase-shifts in response to a light pulse given at the beginning of their rest or active period demonstrated that rods and cones also contribute to this process but only partially [106], since light responses were more marked in animals with rods, cones and ipRGCs.

Circadian photoentrainment is explained by the differential sensitivity of the circadian pacemaker to the resetting effects of retinal light exposure depending on the circadian phase at which light exposure occurs [107]. Bright light exposure induces phase delays of the circadian pacemaker when the light stimulus is applied during the period between midday and the time of minimum core body temperature, while phase advances occur when the light stimulus is administered some time after the point of minimum temperature and prior to midday as shown in figure 3. Minimum body temperature occurs at the beginning of the second part of the spontaneous nocturnal sleep period and shortly after the acrophase of the peripheral skin temperature rhythm or the acrophase of melatonin rhythm. Thus, the circadian system and the sleep of patients can be advanced by light exposure in the morning, but it is delayed by light exposure during the evening and early part of the night [107]. When housed under normal conditions, patients will receive light both in the advanced and delayed zone, and thus the phase of the circadian system will be shifted according to the balance of these two light exposures, weighted according to the phase response curve (PRC).

Although not studied in ICU conditions, it is known that bright light exposure during the critical period (also known as singularity) which occurs around the time of minimum core body temperature is also capable of inducing a prolonged period of arrhythmicity [108, 109]. Light should therefore be avoided, particularly during this period, in order to preserve the circadian rhythmicity of ICU patients.
Light-dark conditions and ICU patients

One of the environmental factors associated with sleep impairment and delirium in ICU patients is their inadequate exposure to light during the daytime and darkness at night. Different studies have reported widely varied data regarding exposure to illumination, with daylight levels between 50 and 400 lux, and night levels of 0-150 lux. In some cases, nighttime peaks were >1000 lux, which is above the threshold to produce a complete inhibition of melatonin synthesis [110]. Unpublished data from our laboratory show a wide range of light-dark conditions, (Figure 4) not only between different ICUs (depending on the presence or absence of natural sunlight and the highly variable levels of nocturnal light), but also within the ICU itself, since patient bed location (e.g., close to windows or to the nurse’s station) affects light exposure, as can be observed in Figure 4. Note that even when the bed is well-positioned with respect to the window, light intensities during the day barely reach 100 lux. In addition, light exposure during the standard rest period (from 23:00 to 7:00) is quite common.
There are few studies addressing light levels or light therapy in ICU patients. They can be grouped according to those considering the winter immune enhancement paradigm, the influence of environmental light on ICU patients and the effect of bright light therapy on critically ill patients.

According to the winter immune enhancement paradigm [111], in environments that undergo seasonal changes in energy availability, selection should favor individuals with an enhanced immune function during the winter (shorter days or a shorter photoperiod), a time of the year with less availability of food and a decreased temperature (thus, with more stressors). Critically ill patients live in a “winter like” condition [112], as energy resources are severely compromised, and furthermore immunity is impaired as the immune system tries to fight off many severe aggressions. In critically ill patients, preserving immunity is essential for a good outcome. The defenders of this theory propose providing longer periods of darkness and less hours of light in the ICU in order to simulate the winter photoperiod. In this sense, in an interesting retrospective study [113], a shorter photoperiod during the month prior to a critical illness was associated with a reduced risk of death in critically ill patients admitted to the ICU. However, a shorter photoperiod in the preadmission was not associated with fewer instances of ICU-acquired-delirium [114].

With regard to the effects of environmental light on ICU inpatients, different authors have tried to demonstrate that an ICU environment with a higher level of natural light could be beneficial.
Nevertheless, one study conducted by Wunsch et al. [115] showed that the presence of a window in an ICU room did not improve the outcome of critically ill patients with a subarachnoid hemorrhage admitted to intensive care. This particular study failed to analyze the levels of room light, and another study by Castro et al. [113], evidenced a nearly complete (99.6%) degradation of natural light from the outside to the ICU bed, with light exposure approaching zero once in the ICU. It should be noted that in this same study, 84% of rooms had a window. Therefore, it is not the presence of a window, rather environmental levels of light that should be analyzed in order to draw an accurate conclusion. In this sense, the study carried out by Verceles et al. [116] showed no relationship between light exposure or room orientation and the outcome of critically ill patients or sedative/analgesic/neuroleptic use. However, the authors reported that only the southern-facing rooms received light exposure with peaks greater than 500 lux (maximum: 600 lux). We must keep in mind that for a biological and chronobiological effect, the minimum intensity needed is 10,000 lux for half an hour or 2500 lux for one hour [117]. Therefore, it is probable that a very low level of environmental light could explain the absence of a positive effect in this study. Another interesting study reported that in patients with severe sepsis, light levels did not entrain the melatonin secretion pattern [62]. However, as the authors mention in the discussion, this study has the same limitation as the aforementioned article, namely, very low levels of environmental light (maximum: 200 lux).

Bright light therapy has therapeutic effects for many types of patients: terminal-stage patients, patients with dementia, depression, sleeping disorders and seasonal affective disorder [118, 119, 120]. Bright light therapy has also been useful for the prevention of delirium in post-operative patients [121], and is also a promising adjunctive therapy in patients with established delirium [122]. Post-esophagectomy ICU patients who received two hours of bright light therapy early in the morning [123] presented lower levels of nocturnal physical and sympathetic activity; the level of arrhythmia and the punctuation on a confusion scale (NEECHAM scale) were also lower in the bright light group, and cases of delirium also tended to be lower in this group, albeit with no significant differences. Another study of postoperative patients [121] obtained lower values on a delirium score and an earlier start of ambulation in the intervention group. An interesting study performed by Barroso and Brinker [124] using a mathematical model of circadian rhythms in critically ill patients theorized that with 6 hours of medium bright light (200 lux), 10 hours of normal light (artificial light in a room) and 8 hours of darkness, patients could reach their maximal circadian rhythm amplitudes in three days. In addition, they demonstrated that darkness was as important as light for circadian rhythms, since even a minimal intensity of light during the theoretical nighttime period considerably reduced the amplitude of the melatonin secretion rhythm. They therefore concluded that a comfortable intensity of bright light during the morning and an assured dark period of night was a sufficient strategy for enhancing circadian rhythms in critically ill patients. Finally, it has been shown that bright light therapy may not be able to effectively regulate melatonin release in critically ill patients [125]. The authors analyzed the nocturnal release of melatonin in response to 1 hour of darkness followed by 1 hour of bright light (>10000 lux) during the night, which produced no response in 15 out of 15 patients with very low levels of melatonin, and in 5 out of 5 patients with high levels of melatonin. However, this study also has its limitations, among which are the short period of measurement of the melatonin levels (just three hours, during the intervention), since the modifications in the secretion pattern of melatonin could be delayed in time. It also lacks a previous and complete recording of the melatonin secretion pattern, which means that the diversity of this rhythm is unknown among the subjects.

In contrast to the lack of studies in adults, there is a growing body of research on light and circadian rhythms in patients hospitalized in neonatology intensive care units (NICU). The first study, conducted by Mann et al. [126] in a newborn nursery, showed that infants from the 24-hour nursery with reduced light and noise during the night period gained more weight than the control group. Boo et al. [127] later compared the weight gain between preterm infants exposed to 12 hours of cyclical lighting and those exposed to a continuously dim light environment; in this study, there were no significant differences between the two groups. Subsequent studies have demonstrated the beneficial effects of a cycled light environment versus an environment with continuous dim light/near darkness on preterm infants, including higher oxygen saturation and lower respiratory rate [128, 129], a greater rate of weight gain [129, 130,
131, 132], a reduction in fussing and crying behavior and a trend toward higher motor activity during the day [131], an earlier beginning of oral feeding, a shorter period of use of ventilators and enhanced motor coordination [132], a shorter hospital stay and an earlier development of a daily melatonin rhythm [129] and a faster acquisition of the rest-activity pattern after discharge [133]. Finally, two studies have analyzed the risk of retinopathy of prematurity and the influence of cycled light [130, 134], revealing no differences between the two groups (continuous patching of eyes and cycled light) in the incidence of retinopathy, which supports the use of this therapy in the NICU. In conclusion, in a review by The Cochrane [135], exposure to cycled light appears to be preferable to continuous light, dim light or near darkness in preterm and low-weight infants, although more studies are needed to establish a clear recommendation. However, the potential usefulness of light therapy in these patients requires that guidelines be determined for its safe application.

**Potential usefulness of light therapy in an ICU context**

The first somatic antidepressant developed from a biological hypothesis was bright light therapy [136]. Bright light therapy was explored in the late 1970s for the very first time as a treatment for seasonal depression [137, 138]. This hypothesis was supported by epidemiological data reporting the high incidence of depression in winter and the idea of lengthening the day to obtain the remission of depression in the winter [138, 139].

However, the establishment of bright light as a therapy was difficult because of the absence of a placebo, due to the fact that the light treatment cannot be concealed. As a result, four comparative approaches were developed to address this issue: 1) bright light versus dim or colored light; 2) duration between half an hour and an hour, versus brief sessions; 3) morning versus evening light exposure; and 4) bright light therapy versus low-intensity or deactivated negative air ionizers [140]. All of these approaches are referred to as “therapy versus placebo”.

Once bright light therapy was established, the therapeutic range of each of the parameters was elucidated. In current practice, prior to bright light therapy, the following parameters must be defined:

**Field of illumination:** The field of illumination should be as large as possible, in order to maintain the therapeutic light level in spite of head movements. The standard 60 cm by 120 cm fluorescent ceiling unit is recommended [141, 142].

**Direction:** Light should be directed from above the eye level, due to the enhanced melatonin suppression with directional illumination of the lower retina [143]. For bed-ridden patients, the ideal treatment would be to emit the light from the headboard of the bed in order to obtain the same relative direction for the eyes.

**Intensity and duration:** Contrasted and recommended intensities vary between 10,000 lux (the highest level verified for safety and efficacy) and 2500 lux, and with durations ranging from half an hour to one hour of treatment, depending on the intensity (higher intensity equates to a shorter duration). This configuration provides better results or permits reducing side effects by modifying the intensity and duration [117, 142].

**Spectrum:** Full spectrum devices are traditionally recommended, with a plastic diffusion screen to block ultraviolet radiation. However, in order to diminish side effects, the low-range blue is also eliminated [117]. Nowadays, soft white fluorescent lights (3000-4000K) are preferred to cool white or full-spectrum lamps (5000K or higher) [142].

**Circadian timing:** The time of day should be focused on twilight, more specifically at the subjective dawn, in order to obtain a stronger circadian effect [142].
Despite all of this, bright light therapy devices and modes of administration remain unregulated. The protocol is easy to implement, but it requires trained clinicians in order to obtain desirable and therapeutic results and prevent side effects [117]. The therapeutic effects of properly administered bright light therapy includes sleep pattern consolidation or resetting of sleep timing, and affective and/or physical status improvements [141, 144, 145, 146, 147, 148].

However, side effects sometimes appear even when everything is properly controlled. These side effects include mild visual complaints (eye irritation or fatigue), hypomania, headache, nausea, dizziness, and insomnia after late evening light and premature awakening after morning light [142]. Fortunately, these symptoms are infrequent or even rare, and they usually subside after several days of treatment [138, 149, 150]. In addition, medications that induce photosensitization to the short wavelength band (the blue band of the spectrum), including psoralen, neuroleptic, porphyrin, antimalarial, antiarrhythmic and antirheumatic drugs [117] must be controlled. Ultraviolet sensitizers are not incompatible with bright light therapy, as diffusers block this spectrum range. Finally, low blue range photosensitizers are also important and less frequently associated with harmful effects. These photosensitizers include diuretics, antidepressants, tetracyclines, or sulphonamides [142]. The last contraindicated factor requires extra attention, as it comprises medical conditions aggravated by bright light, such as retinal dystrophies, age-related macular degeneration, porphryia, lupus erythematosides, chronic actinic dermatitis and solar urticaria [142].

There is a novel method especially suited for intensive care unit patients. It is based in dawn and dusk simulation. For a successful dawn simulation, a configurable and programmable broad field with diffuse illumination from the headboard above the bed is essential [117]. In this therapy, room light levels are very dim, with soft white or blue-blocked light at night. The light level increases from dawn onwards, until reaching the standard room level (around 300 lux). It remains constant until dusk, when the light level is gradually lowered to the night level, producing similar results to bright light therapy [142, 144]. These effects can be explained by the light exposure reception and transmission in the retina through our translucent eyelids [144, 151, 152, 153]. Dawn and dusk simulation is like walking outside into the sun after waking up at sunrise, with the difference that the patient is still sleeping [117], which prevents ocular photosensitization [117].

**Melatonin therapy and critically ill patients: from bench to bed.**

Under environmental conditions where the melatonin rhythm is highly compromised, the use of exogenous melatonin to preserve circadian rhythmicity and to promote sleep, reduce delirium and improve immunological response should be considered. In this sense, some studies reported the ability of the timely administration of exogenous melatonin to synchronize sleep-wake rhythms in totally blind people, a condition that has some similarities to patients in a constant ICU environment. Exogenous melatonin produces an improvement of agitation and sundowning in demented patients [50]. In fact, in two randomized, controlled trials, the administration of exogenous melatonin at low doses (0.5 mg) in institutionalized elderly patients was effective in reducing the risk of delirium [154, 155]; however, there is no evidence to support the specific use of melatonin in critically ill patients, as control trials are still lacking.

Severe sepsis and septic shock represent progressive and the most severe stages of a systemic inflammatory response to infection, which leads to end-organ dysfunction and/or failure [156, 157]. Epidemiological data show that sepsis is one of the main causes of admission to the intensive care unit (ICU), the major cause of death in the non-coronary ICU and a life-threatening complication among hospitalized patients, particularly elderly, immunocompromised and critically ill patients [158, 159]. In spite of its early identification and the significant advances made in ICU treatment, severe sepsis still presents a high incidence (13.3% per year) and mortality (12.1%-25.6%) rates [160, 161]. A better...
understanding of the pathogenesis of sepsis is therefore necessary for the design of new therapeutic strategies to improve the clinical outcome.

Factors that contribute to the pathophysiology of sepsis and represent a promising target for therapy include: (i) improper activation of the inflammatory processes, with an overproduction of pro-inflammatory cytokines, which not only cooperate to amplify both the severity and duration of the sepsis-associated inflammatory response, they also lead to circulatory shock, ischemia and organ dysfunction [162, 163, 164]; (ii) marked oxidative stress, responsible for cellular lipoperoxidation, DNA and protein oxidation, intensifying organ injury and thus worsening the overall clinical outcome [165, 166, 167], and (iii) mitochondrial dysfunction directly associated with an overproduction of reactive oxygen species (ROS) and nitric oxide (NO) derived from mitochondrial nitric oxide synthase (mtNOS), characterized by damage to respiratory complexes, increased ROS/RNS formation, ATP depletion and cell death [168, 169, 170, 171, 172].

Interestingly, the key role of mitochondria in the development of sepsis-associated organ dysfunction, as well as the interplay between pro-inflammatory signalling and mitochondrial dysfunction [173, 174], supports the view that current symptomatic and supportive treatments must be replaced by novel therapies specifically targeting these organella. In addition to its chronobiotic effects, melatonin has well-documented protective effects against severe sepsis/shock in both animal models and septic patients. Melatonin is a powerful free radical scavenger, with antioxidant and anti-inflammatory properties [166, 169, 171, 172]. Given the ability of mitochondria to take up melatonin in a time- and concentration-dependent manner [175, 176], this indoleamine provides in situ protection against oxidative damage at the mitochondrial level. Here, melatonin reduces the production of mitochondrial ROS, inhibits iNOS/i-mtNOS expression and activity, maintains mitochondrial glutathione levels and restores normal respiratory activity and ATP production [166, 169, 170, 171, 172, 177]. Based on these findings, it is not surprising that melatonin treatment improves survival in animals with septic shock (Figure 5) [171].

**Figure 5.** Percent and time of survival of septic mice, with or without melatonin treatment. Sepsis was induced by cecal ligation and puncture (n=20). Redrawn from Escamez et al., 2007 [171].

How does melatonin benefit septic patients? Impaired circadian melatonin secretion has been monitored in severely septic patients through the analysis of urine concentration of 6-sulfatoxymelatonin, and it tends to normalize within 1-4 weeks after recovery from sepsis [59, 62]. Importantly, a clearly negative correlation between disease severity and serum melatonin levels at 2 a.m. was detected in severely septic
patients, but not in ICU patients admitted for other causes, including coronary syndrome, gastrointestinal bleeding or pneumonia [178]. Besides the continuous administration of drugs and the loss of external zeitgebers, desynchronization of the circadian melatonin rhythm has been linked to the overproduction of pro-inflammatory cytokines, such as IL-1 and TNF, which drastically inhibit both melatonin precursor synthesis and transcription of biosynthetic pathway enzymes [179, 180]. Gitto et al. [181] demonstrated for the first time that melatonin treatment improves the clinical course of septic infants. In this study, a total of 20 mg of melatonin was administered orally to septic newborns in two doses of 10 mg each, with a 1 hour interval between them, within the first 12 hours of diagnosis. Due to its anti-inflammatory effects, white blood cell count, absolute neutrophil count and C reactive protein levels, which remain high in untreated septic patients, were significantly decreased by melatonin treatment. Moreover, the antioxidant effects of melatonin were also confirmed by the drop in serum levels of lipid peroxidation products in melatonin-treated septic newborns, as compared to untreated septic newborns. Importantly, 3 out of 10 untreated patients died 72 hours after the diagnosis of sepsis, while all melatonin-treated patients survived. Subsequently, melatonin treatment was also used in newborn patients affected by hypoxia or respiratory distress; in this case, it reduced inflammatory and oxidative stress parameters and improved the clinical outcome [182, 183].

While experimental models have suggested the efficacy of several therapeutic approaches based on preclinical trials, the translation to human septic patients also seems to have been successful. Additional trials in septic adult patients should be carried out to evaluate the efficacy of melatonin therapy in these subjects. The findings summarized here show important therapeutic implications for the potential use of melatonin in septic and critically ill patients. However, the existence of mechanisms capable of regulating the intracellular concentration of melatonin supports the requirement for high doses of melatonin to obtain beneficial therapeutic effects [176]. In any case, a variety of studies have shown that melatonin not only had no significant toxicity [184, 185, 186], it was also rapidly cleared at high doses following oral administration, preserving its beneficial effects on sepsis-induced mitochondrial dysfunction, oxidative stress and pro-inflammatory response [187].

**Chronoenhancement through increasing day-night contrast in the ICU environment.**

There is little doubt that intensive care units are invaluable for the treatment of patients with acute organ failure. However, there is a price to pay on the part of the patient in terms of physical and psychological discomfort as an unavoidable consequence of testing, monitoring and therapeutic interventions, as previously discussed. But other factors related to the ICU environment may be modifiable.

Throughout this review, we have evidenced the chronodisruptive effects of environmental constancy, but little is known about how to chronoenhance the circadian system. In theory, increasing differences between daytime and nighttime exposure to environmental light and temperature, activity, posture and sleep should strengthen the robustness of the circadian system [188], but some of these measures are not feasible in ICUs. Nonetheless, attempts have been made to control the ICU environment, including noise reduction and controlling light exposure.

Numerous studies have found excessive noise levels [82] from different sources, including ventilation, alarms, phones, beepers and conversation. They are far above 45dB during the day and 35dB at night [67], which exceeds the recommendations from the Environmental Protection Agency for peak noise levels. However, their relative contribution to CD is not easy to assess. Some studies [70, 189] reported that although noise reduction improved sleep quality in ICU patients, the awakening index or arousals were not modified, indicating that this intervention was not efficient enough to improve sleep. However, other authors [190, 191] found an improvement of almost all sleep parameters in healthy volunteers under a simulated hospital environment who wore earplugs at night. Also, a “quiet time” program developed by Dennis et al. [192] demonstrated lower levels of light and noise, and a greater probability of sleep during the quiet hours. In any case, controlling noise exposure “at the patient’s will” is not an easy feat in the
IUC, but what can be done is to simply reduce talking, especially during the night, since substantial noise is caused by the staff members themselves [193]. A simple behavior modification has been found to attenuate sound peaks in these units [194]. Other practicable measures are setting pagers to vibrate rather than ringing and reducing the number of unnecessary alarms [195, 196], reducing staff congregation in order to socialize, and educating them in the negative effects of sound [197].

Whether light exposure directly affects the incidence of delirium is not known, but reduced light exposure has been cited among the precipitating factors [114, 198]. However, the importance of light as a disruptor of patients’ sleep and circadian physiology may vary depending on the light level in each ICU (windowless, same intensity during day and night, etc.). Accordingly, the first countermeasure should be to monitor the 24-h light pattern in the same ICU in order to determine how much light there is and, most importantly, when it is received, as opposed to the use of weather stations to determine the photoperiod, as in previous studies [114].

A cycled light system that tries to mimic the natural day and night light rhythm has been reported to be beneficial in terms of producing earlier day/activity and night/rest rhythm in the NICU settings, but there is a lack of research on how adult ICU patients may be affected [199]. In addition, blocking or excluding blue light during the night could minimize the adverse effects on melatonin nocturnal surge and contribute to the maintenance of circadian cycles.

Other studies have analyzed the effect of “night protective elements” on ICU patients. Reducing both light and noise during the night through the use of earplugs and eye masks decreases arousals and increases sleep quality [36, 82], as well as producing higher melatonin levels in urine [82, 200], but their effects on delirium outcomes still need to be evaluated [196]. Nevertheless, it has been proposed as a comfortable, tolerable and cheap intervention that should be offered to patients by nursing staff.

Few attempts have been made to minimize the impact of chronodisruption on the health of patients in intensive care units by enhancing day-night contrast according to a multifaceted approach 52, 201, probably due to more urgent life-threatening concerns. The ICU is a potentially hostile environment that should be transformed in an optimal healing milieu [196].

When a multicomponent bundle of interventions is considered, the occurrence and duration of delirium can be significantly reduced in conjunction with an increase in both qualitative and quantitative sleep indexes [201].

These interventions (Figure 6) include reducing the volume on telephones, earplugs, light timers that turn them off and on, dim nocturnal light, raising window blinds, eye masks, grouping patient care activities when possible, appropriate sedation [52, 201], early mobilization and even ensuring patient orientation with respect to time [67, 195]. This latter, and again simple, recommendation has been included in the National Institute for Health and Clinical Excellence Guidelines for the Prevention of Delirium [202], “ensuring that a 24-hour clock and a calendar are easily visible to the person at risk”, “and facilitating regular visits from family and friends” (in other words, regular social contacts).

In addition, Patel et al. [201] reported a 90% compliance rate with the scheduled interventions by the staff, probably as a consequence of their being included in the staff education program, with training sessions on the task to be performed, as well as background information on sleep and delirium, which surely contributed to compliance and the subsequent success of the actions. In fact, the importance of increased staff awareness on the effectiveness of the intervention has previously been highlighted [202].

Finally, real time non-invasive monitoring of the ICU environment that could provide feedback to caregivers on those variables that should be corrected (light, noise, temperature, etc.), in addition to a smart system that registers the circadian rhythms (actimetry, skin temperature, humidity, etc.) of the patients in bed, could interact to automate some of the most important interventions required to minimize circadian disruption in the ICU, rendering it a more “optimal healing environment”.
Figure 6. Summary of measures that can be applied to enhance the biological rhythms in an ICU patient. Each attempts to increase the contrast between day and night, in order to decrease circadian disruption, which frequently afflicts patients, as well as those pathological conditions that can be exacerbated or triggered by this hostile environment.

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Conflicts of Interest

The authors declare no conflicts of interest.
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