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## Emerging relevance of circadian rhythms in headaches and neuropathic pain

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### Abstract

Circadian rhythms of physiology are key to health and fitness, as dysregulation, by genetic mutations or environmental factors, increases disease risk and aggravates progression. Molecular and physiological studies have shed important light on an intrinsic clock that drives circadian rhythms and serves essential roles in metabolic homeostasis, organ physiology, and brain functions. One exciting new area in circadian research is pain, including headache and neuropathic pain for which new mechanistic insights have recently emerged. For example, cluster headache is an intermittent pain disorder with an exceedingly precise circadian timing, and preliminary evidence is emerging linking several circadian components (e.g., *Clock* and *Nr1d1*) with the disease. In this review, we first discuss the broad metabolic and physiological relevance of the circadian timing system. We then provide a detailed review of the circadian relevance in pain disease and physiology, including cluster headache, migraine, hypnic headache, and neuropathic pain. Finally, we describe potential therapeutic implications, including existing pain medicines and novel clock-modulating compounds. The physiological basis for the circadian rhythms in pain is an exciting new area of research with profound basic and translational impact.

### Keywords

circadian rhythms; chronotherapy and clock-modulating compounds; cluster and hypnic headaches; metabolism; migraine; neuropathic pain

### Introduction

An internal 24-hour timer, or circadian clock, is present in organisms ranging from bacteria to plants to animals<sup>1, 2</sup>. It coordinates essential functions, and in doing so conserves energy or promotes activity at the appropriate times<sup>3</sup>. Examples include body temperature (highest before midnight), blood pressure (highest at midday), pulmonary capacity (forced expiratory volume in one second or FEV1 highest in the afternoon), and sleep (tiredness is highest around midnight). Understanding these basic circadian functions has led to simple but

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important medical advances in chronotherapy, namely drug administration at specific circadian times. For example, blood pressure medications are given before the blood pressure peaks<sup>4</sup>, and asthma medications are given before the pulmonary function declines<sup>5, 6</sup>.

The internal circadian clock also affects metabolic homeostasis<sup>7, 8</sup> and higher-order functions<sup>9, 10</sup> such that there are peak times every day for gluconeogenesis, schoolwork, and athletic performance. Thus when the circadian system is disrupted, it increases the risk of a wide range of diseases. Animal and human studies have shown that debilitating mutations or disruptions of core circadian genes can have adverse effects on metabolic, physiologic and neurologic processes<sup>11, 12</sup>. For example, mutations in the human circadian gene Casein Kinase (Ck) 1delta lead not only to sleep cycle shifts, but also to an increased incidence of migraines<sup>13</sup>. Understanding these complex circadian functions, and their role in human disease, has the potential to lead to significant medical advances. In this article we review the role of circadian rhythms in physiology, and in particular highlight pain as an emerging field for circadian research.

## Circadian clock and its broad relevance in physiology

The circadian cycle is present at the single cell level: individual cells grown *in vitro*, such as fibroblasts, are capable of generating a circadian rhythm<sup>14, 15</sup>. Within the cell, the clock machinery is a series of intersecting transcription-translation feedback loops that activate and inhibit each other in a cycle lasting about 24 hours<sup>16</sup> (Figure 1). These core circadian genes encode both positive (CLOCK: Circadian Locomotor Output Cycles Kaput; NPAS2: Neuronal PAS domain protein 2; BMAL1: Brain And Muscle ARNT-Like 1; ROR $\alpha/\beta/\gamma$ : Retinoid acid-related Orphan Receptor a, b, c) and negative (PERIOD1/2/3, or PER1/2/3; CRYPTOCHROME1/2, or CRY1/2; REV-ERB $\alpha/\beta$ : Reverse strand of erb) proteins. Additional factors have been identified, which either form auxiliary loops or regulate the core clock components<sup>16, 17</sup>. The core oscillator subsequently regulates tissue-specific expression of output genes via elaborate genetic and epigenetic mechanisms<sup>18</sup>.

On a larger scale, the circadian rhythm is present throughout the body: organ explants isolated *in vitro* show a circadian rhythm regardless of tissue type, as brain, liver, lung, kidney, and other tissues have shown circadian rhythmicity<sup>19</sup>. Even though each cell has its own rhythm, cells of a single organ typically function together as a single circadian unit called a peripheral clock. These peripheral clocks run independently but are synchronized by a central pacemaker – the suprachiasmatic nucleus (SCN)<sup>20</sup>. The SCN was shown as the central clock in elegant experiments where the SCN was either lesioned (abolishing the 24 hour cycle) or where the SCN in a wild-type hamster was replaced with a mutant SCN with a 20 hour cycle (and the wild-type hamster began to display a 20 hour cycle)<sup>21–23</sup>. Located in the anterior hypothalamus, the SCN is a remarkably robust self-sustaining oscillator<sup>24</sup>. Both the SCN and peripheral clocks can be calibrated, or entrained, by various stimuli called zeitgebers (time givers)<sup>25</sup>. Light is the predominant zeitgeber and is relayed to the SCN via the retinohypothalamic tract. Light enters this tract not by rods or cones but by a third group of photosensitive cells, the photosensitive retinal ganglion cells, which contain melanopsin and use glutamate and pituitary adenylate cyclase-activating peptide (PACAP)<sup>26, 27</sup>. Food

can also entrain body clocks: when light is kept constant over 24 hours, and thus is eliminated as a zeitgeber, rats exposed to food once a day will entrain to that food stimulus<sup>28</sup>. Other zeitgebers for the SCN and peripheral clocks include temperature, exercise, and circadian hormones (i.e., steroids and melatonin)<sup>29–31</sup>. The SCN communicates with peripheral clocks through circadian hormones, and communicates with other brain areas through neuronal connections. SCN neurons project to various locations in the thalamus, hypothalamus, and basal forebrain<sup>25</sup>. These include areas such as the periventricular nucleus of the hypothalamus and the nucleus of the vagus<sup>32</sup>, which are critical areas of the autonomic nervous system. It may also connect to the lateral hypothalamus, which contains orexin and is important in sleep regulation.

Commonly used biomarkers for the circadian system are the hormones melatonin and cortisol. Melatonin is a biological marker of darkness, rising at dusk and declining before dawn, and a light pulse will acutely reduce the production of melatonin. Melatonin is produced and released primarily by the pineal gland, which is directly controlled by the SCN via sympathetic neurons. Melatonin interacts with most if not all cell types and may be one way that the SCN synchronizes all of the peripheral clocks<sup>30, 33</sup>. Melatonin may be used as a measure of the yearly cycle: the duration of melatonin release is a marker of day length, and day length varies from the summer to the winter solstice. Corticosteroids, in contrast, are a biological marker of day and display a pattern opposite to melatonin. Like melatonin, corticosteroids synchronize the peripheral clocks and are often used for this purpose experimentally<sup>34</sup>: *in vitro*, individual cells each display a slightly different circadian cycle, and dexamethasone is used to synchronize the cycles of an entire cell population. Thus either by direct neuronal connections from the SCN or by hormonal communication, the entire circadian system can be synchronized.

The majority of all genes are cyclically expressed in at least one organ in mammals<sup>35, 36</sup>. Affirming the physiological importance of the circadian clock, the subset of genes expressed in each tissue often encompasses regulatory genes for various physiologies associated with organ function<sup>37</sup>. For example, in a transcriptional cascade required for myocardial repolarization, CLOCK/BMAL1 activates *Klf15* gene expression; subsequently, KLF15 proteins activate expression of the gene encoding the cardiac ion channel component KvCHIP2<sup>38</sup>. Furthermore, PER2 proteins were found to be stabilized by adenosine signaling in response to myocardial ischemia, and play an important role to reprogram hypoxic and metabolic pathways<sup>39</sup>. These circadian changes in the cardiovascular system may be relevant for human medicine. In a recent study, patients were randomized to morning or afternoon surgery for aortic valve replacement. Improved surgical outcomes were found in the afternoon group and were linked to changes in the expression of the core circadian component REV-ERB $\alpha$ <sup>40</sup>.

Finally, circadian patterns are not the only type of cyclical pattern in humans, and hormones and transcriptional-translational feedback loops are not the only mechanism for generating them. Ultradian rhythms refer to cycles that are shorter than 24 hours: an example is heart rate, which cycles 70–100 times per minute in a resting human and is controlled by the nervous system. In contrast, infradian rhythms have a cycle that is longer than 24 hours.

Infradian cycles can be hormonal such as the monthly menstrual cycle, or behavioral such as the weekly pattern of sleep that changes from the workweek to the weekend.

## Circadian rhythms in pain

The most common reason to seek medical care is pain, and patients with a variety of pain disorders have reported distinct daily rhythms of pain intensity<sup>41–43</sup>. A well-known pain disorder with circadian timing is rheumatoid arthritis: morning stiffness and morning pain have been described for over a half-century, with morning stiffness proposed as a diagnostic criterion in the 1950's<sup>44</sup>. Recent studies have linked arthritic morning pain to the daily cycle of inflammatory cytokines<sup>45</sup>. Moreover, there appears to be a role for chronotherapy of immunosuppressants in treating rheumatoid arthritis<sup>45</sup>. The circadian biology of other pain disorders is also emerging. Below, we will focus on headaches (including cluster headache, migraine, and hypnic headache) and neuropathic pain (chronic pain after nerve injury) for which new mechanistic information has emerged (Figure 2).

## Cluster headache

Cluster headache affects 1 in 1000 people and starts between the ages of 20–40, with a 3:1 ratio of male to female<sup>46–48</sup>. It is often described as one of the most painful human experiences and has been nicknamed “suicide headaches,” as over 50% of patients have contemplated suicide<sup>49, 50</sup>. Cluster headache is characterized by unilateral attacks of exquisite pain lasting 15–180 minutes, occurring between once every other day and eight times per day, and is associated with restlessness (patient pace during a headache) and autonomic changes of the face (like a bloodshot or watering eye)<sup>51</sup>. The vast majority (90%) of cluster headache patients will have headaches every day for weeks-to-months and then will have no headaches for several months, as if the headaches are clustering together. This cyclical pattern is termed episodic cluster headache; the remaining 10% of patients have chronic cluster headache with no headache-free period lasting longer than three months. The exact mechanisms of cluster headache are not known, but current research from imaging and animal studies suggests that three systems are involved. First, the trigeminovascular pain system receives inputs from the meninges and large cranial blood vessels and transmits information to the trigeminal nucleus and the dorsal horn of the adjacent three cervical levels (C1–3). Second, the autonomic system connects from the superior salivatory nucleus to the sphenopalatine ganglion and is thought to be responsible for the cranial autonomic features of the disease. Third, imaging data suggests hypothalamic activation during a cluster headache attack<sup>52</sup>. In this imaging study, nine cluster headache patients and eight controls were exposed to nitroglycerin, which induced a cluster headache attack in the cluster headache subjects but not in the control subjects. Positron emission tomography (PET) was performed at baseline, during nitroglycerin infusion, during the headache, and after the headache. The ipsilateral hypothalamus showed increased regional cerebral blood flow only during the headache period and only in the cluster headache group. The hypothalamus contains several areas with potential roles in cluster headache, including the central clock (the SCN) and an area anterior and medial to the fornix that has been implicated in defensive rage and might explain the restlessness and self-aggression seen in the disease<sup>53, 54</sup>.

These three systems – the trigeminovascular pain system, the autonomic system, and the hypothalamus – appear to be strongly interconnected in cluster headache patients. Deep brain stimulation of the hypothalamus is a proposed treatment for cluster headache based on preliminary studies<sup>55, 56</sup>, and cluster headache patients with implanted hypothalamic deep brain stimulators show higher cold pain thresholds<sup>57</sup>. Pain thresholds in general, such as the nociceptive flexion reflex in the leg, show circadian variation in episodic cluster headache patients compared to controls or chronic cluster headache patients<sup>58</sup>. Modulation of the autonomic system via the sphenopalatine ganglion can induce or relieve cluster headache pain depending on the stimulation setting, and stimulation of the superior salivatory nucleus in rodents activates neurons in the trigeminal nucleus<sup>59</sup>. One clear link between the circadian system and the mechanisms of cluster headache is that the central clock (the SCN) resides in the hypothalamus.

One hallmark feature of cluster headache is its prominent circadian and circannual patterns<sup>60</sup>. Patients generally have headaches at precisely the same time every day, and have headache cycles at precisely same time every year or every other year. For example, a typical patient may say that he has an attack at 2 AM every day April, then is headache-free until the following April. Another may say she has headaches at 10 PM and midnight every night in autumn, though it will sometimes skip a year. A large study of 1134 patients showed that 82% of cluster headache patients have headaches at the same time every day<sup>49</sup>. The most common time of day for an attack was 2 AM in three large studies<sup>49, 61, 62</sup>, and the most common times of year appear to be spring and autumn. Possibly the most strikingly circadian example reported is a Danish woman who underwent a sleep study over 2 nights and had 9 headaches, each almost exactly 90 minutes apart with one exception where she awoke but did not have a headache<sup>63</sup>. Several circadian zeitgebers can trigger an extra cluster headache attack, including exercise and temperature<sup>49, 64</sup>. Alcohol is the most common cluster headache trigger<sup>49, 62</sup>, and ethanol is known to disrupt multiple aspects of the circadian cycle<sup>65</sup>. Likewise, an overwhelming number of cluster headache patients smoke<sup>66–68</sup>, and nicotine has been reported to entrain the circadian clock<sup>69</sup>. Interestingly, these triggers work only during a headache cycle, and do not trigger a headache in the headache-free cycle, suggesting a circannual pattern of neural sensitivity.

The circadian properties of cluster headache extend beyond behavior, as cluster headache patients demonstrate alterations in key components of the circadian system. Several studies have noted changes in melatonin rhythms in cluster headache patients, including an overall decrease in melatonin levels, abolished daily and nightly rhythms of melatonin, and desynchrony between melatonin and cortisol<sup>70–72</sup>. In episodic cluster headache patients, these melatonin changes appear to be more pronounced during a headache cycle than during a headache-free cycle<sup>71, 73</sup>. Cortisol levels are also dysregulated in cluster headache<sup>70, 73</sup>. Furthermore, there are abnormal levels of vasoactive intestinal peptide (VIP) and arginine vasopressin (AVP) in cluster headache<sup>74, 75</sup>. VIP and AVP are peptides found in a large proportion of SCN neurons: VIP-positive neurons are primarily found in the core area of the SCN, and AVP-positive neurons are found in the shell area<sup>20</sup>. Preliminary studies also suggest cluster headache-associated changes in other molecules indirectly modulated by the SCN, including PACAP, orexin, orexin receptors, testosterone, prolactin, and growth hormone<sup>76–82</sup>.

Although earlier attempts did not identify a molecular association of clock genes with cluster headache<sup>83–85</sup>, more recent studies elucidated changes in circadian transcription-translation feedback loops. In an association study of large Swedish cohorts, a single nucleotide polymorphism (SNP: rs12649507) in the *Clock* gene was found to be significantly associated with cluster headache<sup>86</sup>. Although *Clock* mRNA expression was similar in primary fibroblasts between healthy controls and cluster headache patients, the disease-associated SNP above was found to increase mRNA expression. In another study of 8 cluster headache patients who responded to lithium as a treatment and were currently or recently in a headache cycle, lymphoblasts were immortalized with Epstein-Barr virus and subjected to microarray analysis. Compared to 10 healthy controls, cluster headache patients showed markedly decreased expression of the core circadian gene *Nr1d1* (encoding REV-ERB $\alpha$ , a circadian transcription repressor)<sup>87</sup>. Interestingly, the most significantly altered gene in cluster headache in this study was *RBM3* (encoding RNA binding motif protein 3). RBM3 is a cold-induced RNA binding protein that has previously been shown to regulate alternative polyadenylation and thereby circadian gene expression level and amplitude<sup>88</sup>. These findings provide initial evidence for alterations in the transcription-translation feedback loops, highlighting potential mechanistic functions of the core clock and regulatory genes *Clock*, *Nr1d1* and *RBM3*. In summary, cluster headaches appear to be integrated with the circadian system at multiple levels including behavior, neuroanatomy, molecular biomarkers, and transcriptomics.

## Migraine

Migraine is a very common headache disorder, as 17.6% of females and 5.7% of males have at least one migraine per year<sup>89</sup>. It is also the 6<sup>th</sup> most disabling disease worldwide<sup>90</sup>. Migraine is characterized by throbbing, moderate-or-severe unilateral pain, lasting 4–72 hours and associated with features such as nausea, vomiting, and sensitivity to light, noise, and motion<sup>51</sup>. The pain is thought to be related to the same trigeminovascular system discussed above. Similar to cluster headache, migraine is accompanied by an early activation of the hypothalamus: the pain is preceded by 1–2 days of a “premonitory phase” of irritability, yawning, and other features that is thought to originate in the hypothalamus, though the exact location in the hypothalamus is unclear<sup>91</sup>. Other mechanisms of migraine include the trigeminovascular system (similar to cluster headache) and the pain neuromatrix. In particular, the hypothalamus and the dorsal rostral pons have traditionally been thought of as migraine generators, though more recent evidence suggests a more complicated mechanism<sup>92</sup>. Thus like cluster headache, migraine patients show early activation of the hypothalamus, which contains the central biological clock.

Migraines are rhythmic at several levels. On a circadian time scale, migraines peak in the morning and midday<sup>93, 94</sup>. In a recent study, migraine subjects who were “morning larks” (i.e. go to sleep early and wake up early) or “night owls” (i.e. go to sleep late and wake up late) were more likely to have migraines in the morning or in the afternoon/evening, respectively<sup>95</sup>. Overall there were more migraines in the mornings because migraine patients had a higher likelihood of being morning larks than controls. Other rhythmic features include a weekly cycle (less common on Sundays)<sup>96</sup>, a monthly cycle tied to menses and estrogen levels<sup>93, 97, 98</sup>, and even a yearly cycle where migraines are more common in the

spring and fall<sup>93, 99</sup>. Behaviorally, migraines can be triggered by stress, menses, alcohol, weather changes, and skipping meals, as well as possibly bright lights and sleep disturbances<sup>100, 101</sup>. Many of these triggers are related to circadian zeitgebers, including the stress hormone cortisol, the neuropeptide PACAP involved in light entrainment, and meal timing<sup>102, 103</sup>. Indeed, eating at a specific period of time (late at night) was associated with reduction in the odds of a headache<sup>104</sup>. At the molecular level, multiple changes have been noted in melatonin. First, patients have lower levels of melatonin on days with a headache than days when they are headache-free<sup>105</sup>. Second, when triggered by a light pulse, melatonin suppression is more pronounced in migraine patients than in controls<sup>106</sup>. Third, in patients with chronic migraine, which is defined as 15 or more days of headache per month for at least 3 months, a variety of nighttime changes in melatonin have been found, including decreased nocturnal melatonin, decreased melatonin in REM sleep, and a delayed nocturnal melatonin peak<sup>71, 107</sup>.

Genetically, there have been several individual gene mutations linked to migraine. Two families with migraines have been identified with distinct missense mutations in the circadian gene *Ck1delta*; the affected subjects also display advanced sleep phases<sup>13</sup>. The latter finding is consistent with previous studies where *Ck1delta* mutations can lead to familial advanced sleep phase syndrome<sup>108</sup>. The best known genetic causes of migraine are three forms of familial hemiplegic migraine, which are linked to three separate ion channel mutations<sup>109</sup>. Interestingly, a mouse model of familial hemiplegic migraine type I has been created, and these mice display signs of an overactive circadian system such as increased wheel-running activity and shifts of *in vivo* SCN activity<sup>110</sup>. Migraine displays circadian behavioral and anatomical features, and a direct genetic connection has been identified in a subset of patients.

### Hypnic headache

Hypnic headache is a very rare headache disorder, with only a few hundred reported cases in the literature. The symptoms usually begin after the age of 50. Also termed “alarm clock headaches,” these headaches occur only during sleep, last 15–240 minutes, and have no autonomic features or restlessness<sup>51</sup>. These headaches typically occur between 1–3 am, and in one study over half of patients had headaches between 2–3 am<sup>111</sup>, which coincides with the peak of cluster headache attacks. Like cluster headache and migraine, alcohol may be a trigger in hypnic headache<sup>112</sup>. The pathophysiology of hypnic headaches is poorly understood, but it has been postulated that the disease is either a circadian or a REM sleep disorder<sup>112</sup>. Several studies have suggested that the disorder originates in REM sleep<sup>113, 114</sup>, though there is evidence that it occurs in other sleep stages as well<sup>115, 116</sup>. Melatonin levels do not appear to differ in hypnic headache patients compared to healthy controls<sup>117</sup>. However, medications that alter the circadian cycle have been effective in hypnic headache, such as lithium, melatonin, and caffeine<sup>51</sup>.

### Other headache syndromes

Cluster headache, migraine, and hypnic headache are not the only headache / facial pain syndromes that have connections to the circadian system. Sinusitis has a peak in several symptoms (sneezing, congestion, runny nose) at 6 AM, and chronotherapy of histamine 1

receptor blockers has been proposed<sup>118</sup>. Patients with temporomandibular disorder show evidence of increased daytime cortisol, altered nocturnal heart rate variability, and possibly daily variation in pain<sup>119, 120</sup>. Limited circadian data does exist for other disorders such as tension-type headache (altered melatonin levels)<sup>71</sup> and burning mouth syndrome (pain worsening in the evening)<sup>121, 122</sup>.

### Chronotherapy for headache disorders

Various behavioral, dietary and environmental strategies have clear circadian effects. Bright light in the morning, meal-timing and melatonin are commonly employed in clinical trials to reset circadian rhythms<sup>123</sup>. Melatonin and melatonin receptor agonists have also been tested in jetlag, sleep and other neurological disorders<sup>33, 124</sup>. Melatonin and a number of compounds have also been applied to pain<sup>125</sup>. For cluster headaches, there are currently nine preventive medications available in the United States that are recommended by either American or European headache guidelines<sup>126, 127</sup> (Figure 2), including melatonin and corticosteroids. Several other cluster headache preventive medications also have circadian effects. Lithium, also a commonly used mood stabilizer, inhibits GSK-3 $\beta$  and alters clock protein abundance and oscillation<sup>128, 129</sup>. The antiepileptic valproate results in phase-shifts of PER2 reporter rhythms, and the GABAB agonist baclofen can reset the SCN *in vitro*<sup>130, 131</sup>. Verapamil, a calcium channel blocker commonly used in migraine and cluster headache, can alter circadian rhythm of blood pressure, and is often applied as a chronotherapy<sup>132</sup>. Other pain medications, including NSAIDs and opioids, can also be applied in chronotherapeutic regimens<sup>133</sup>. Melatonin and valproate, and perhaps steroids to a certain degree, have also shown efficacies in migraine prevention, whereas lithium and baclofen do not appear to be effective<sup>134</sup>. In summary, the circadian system appears to play a role in headache at the behavioral, molecular, and treatment levels, and is an enticing area for future study.

Significant efforts have also been dedicated to development of novel clock-modulating small molecules<sup>135</sup>. In several recent studies, such compounds, either naturally occurring or synthetic, have shown promising efficacies in rodent models for various diseases including metabolic disease, mood disorder, and aging<sup>135, 136</sup>. For emerging clock-related diseases such as pain, establishment of model systems will allow testing of novel therapeutics. As mentioned above, the critical next step is to identify the key circadian characteristic or factors that may be causal or serve as biomarkers for the disease. For example, while attenuation of circadian oscillation (amplitude reduction) is characteristic of many chronic diseases and aging<sup>137</sup>, it will be interesting to investigate whether circadian gene oscillation is in fact enhanced in cluster headache. Studies described above have implicated several circadian genes in pain, including *Clock* and *Nr1d1* for cluster headache and *Ck1delta* for migraine (Figure 2). Endogenous and synthetic ligands for REV-ERB $\alpha$  have been extensively studied<sup>138</sup>, and several ligands have in fact shown efficacies to modulate brain functions in mouse models<sup>136, 139</sup>. CK1 $\delta$  plays an important role in PER protein turnover and multiple screening studies have revealed a wide variety of compounds targeting CK1 $\delta$  (or its close homolog CK1 $\epsilon$ ) to alter circadian periodicity<sup>140–143</sup>. Small molecules targeting other components of the circadian feedback loops have also been described<sup>144, 145</sup>. Collectively, this small-molecule toolset can be applied to human cells or mouse disease



models, with the ultimate goal to evaluate their potential efficacies to mitigate circadian-related symptoms.

### Neuropathic pain

Neuropathic pain is pain caused by a lesion or disease of the somatosensory system and is generally a burning, electric, or shooting pain. Neuropathic pain can occur both in the central and the peripheral nervous system: central neuropathic pain includes thalamic strokes and spinal cord injuries, while peripheral neuropathic pain includes painful diabetic peripheral neuropathy, chemotherapy-induced peripheral neuropathy, post-herpetic neuralgia, and trigeminal neuralgia<sup>146</sup>. Mechanistically, the gate theory of pain was first proposed in the 1960s and considers pain a balance between inhibitory and excitatory inputs as well as between painful and nonpainful sensory inputs<sup>147</sup>; chronic pain, then, would suggest an imbalance. One of the hallmarks of neuropathic pain is a shift in pain sensitivity, including allodynia and hyperalgesia<sup>148</sup>. Allodynia is pain from a non-painful stimulus, such as pain when putting on socks in painful diabetic peripheral neuropathy. Hyperalgesia is increased pain from a painful stimulus, such as exquisite pain with a light pinch. When a shift in pain interferes with basic activities like putting on socks, significant disability can occur.

Daily patterns in nerve pain have been well-described. Electrical stimulation of the sural nerve, for example, is most painful in the late evening and early morning<sup>149</sup>. Multiple studies have reported that pain from diabetic peripheral neuropathy and post-herpetic neuralgia worsen throughout the day and are worst at night<sup>150–152</sup>. An animal model of neuropathic pain has mirrored these findings. For nocturnal rodents, the hypothesis would be that neuropathic pain in rats would be worse during the day. Indeed, when the sciatic nerve is surgically ligated, rats and mice have increased pain sensitivity during the day<sup>153, 154</sup>. Pain rhythmicity in other experimental models<sup>155, 156</sup> remains to be investigated.

There are multiple proposed mechanisms for the circadian variation of neuropathic pain. Daily variations of mu opioid receptors,  $\beta$ -endorphin, and naloxone response have been reported in rodents<sup>149, 157, 158</sup>. Circadian fluctuations have also been noted in calcium channels involved in pain, as well as in inflammatory modulators<sup>159–161</sup>. In the aforementioned studies of sciatic nerve injury, adenovirus-mediated alteration in the NR2B-CREB-CRTC1 signaling pathway was shown to improve pain behavior<sup>153</sup>, whereas glucocorticoid-induced ATP release from spinal astrocytes aggravates daily hypersensitivity<sup>154</sup>. Changes in the circadian expression of melatonin receptors in the hypothalamus were observed in rodents after peripheral nerve injuries, suggesting a possible role of the central clock<sup>162</sup>. Pioneering studies have further provided molecular evidence that core circadian genes may play an important role in pain. For example, CLOCK:BMAL1 was shown to drive circadian transcription of the *Tac1* gene which encodes the pain signaling molecule substance P in the dorsal root ganglia, suggesting a direct molecular mechanism linking the circadian oscillator and neuropathic pain<sup>163</sup>. Furthermore, partial sciatic nerve ligation in mice was found to suppress circadian oscillation of *Per* genes, and knock-down of *Per1* in the spinal cord by intrathecal injection of siRNA induced mechanical hypersensitivity<sup>164</sup>. In summary, neuropathic pain in general, and diabetic peripheral

neuropathy and post-herpetic neuralgia specifically, show circadian alterations concordant with oscillations in receptors, ion channels, and small molecules that modulate nociception.

## Future directions and conclusions

Circadian rhythms are clearly important for physiological health. In addition to well-established roles in sleep, mood, metabolism and cardiovascular functions, the role of circadian regulation in other organ systems, systemic processes, and gut microbiota is increasingly appreciated<sup>3</sup>. The key challenge is to delineate causal events where circadian changes at the molecular level (e.g., transcription-translation feedback loops) can be propagated to physiological and pathophysiological adaptation. Given the circadian rhythmicity in pain, it is imperative to establish experimental systems, including cultured cells (e.g., human fibroblasts) or rodent models, to delineate key circadian components or focal events that can be targeted for therapy. For example, it will be interesting to investigate a potential functional mechanism whereby the *Clock* gene influences circadian and physiological functions in cluster headache patients. On the one hand, the cluster headache-associated *Clock* SNP (rs12649507) appears to correlate with increased *Clock* mRNA expression in a limited set of samples<sup>86</sup>. On the other hand, whereas genetic ablation of *Clock* in mice leads to diabetes<sup>11</sup>, recent evidence from two large studies shows a decreased prevalence of diabetes in cluster headache patients<sup>66, 67</sup>. Future studies should investigate whether enhanced *Clock* gene expression may contribute to the precise circadian timing while diminishing diabetes risk in cluster headache.

It will be interesting to integrate headache and circadian models in the laboratory for a better understanding of the interactions between pain and circadian rhythms. Multiple models are currently utilized to further understand the pathways and molecules involved in headache disorders. To study the trigeminovascular pain system that may underlie many headache disorders, a commonly used model involves experimental irritation of the dura in rodents via chemical or electrical stimulation<sup>165</sup>. For cluster headache in specific, a rodent model with a lesion of the superior salivatory nucleus has yielded important insights into possible autonomic effects of medications such as oxygen gas<sup>59</sup>. In migraine, two mouse strains have been created as discussed in the migraine section above: the *Ck1delta* human mutation and the *SCN1A* mutation in patients with familial hemiplegic migraine type I<sup>108, 110</sup>. Other animal models have been investigated for temporomandibular disorder such as connective tissue genetic mutations and partial temporomandibular joint discectomy<sup>166</sup>. Among circadian knockout mouse models, *Per1* and *Per2* KO mice have been reported to display altered pain behavior. Specifically, stress-induced antinociception in *Per1* KO mice was partially-reversed (mechanical sensitivity) or over-reversed to hyperalgesia (thermal sensitivity) compared with WT mice<sup>167</sup>. In a morphine-induced tolerance paradigm, *Per2* KO mice developed less tolerance and showed attenuated withdrawal compared to WT<sup>168</sup>.

In conclusion, circadian rhythms permeate many aspects of physiology and pathophysiology. We describe here an emerging link between circadian timing and pain. With a detailed knowledge of circadian mechanisms available, studies of circadian regulation of pain should yield important new insights that can be translated to actionable regimens to alleviate or manage pain symptoms.

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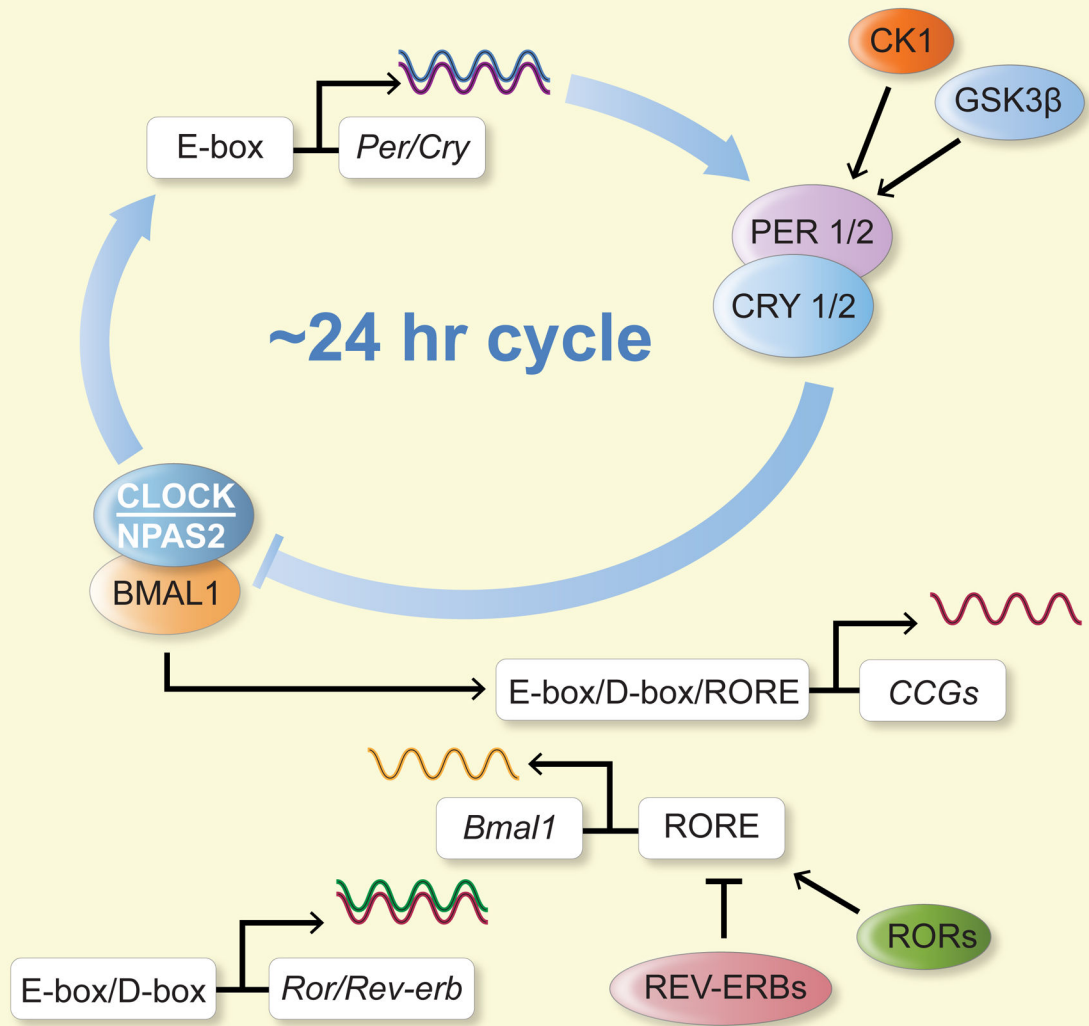
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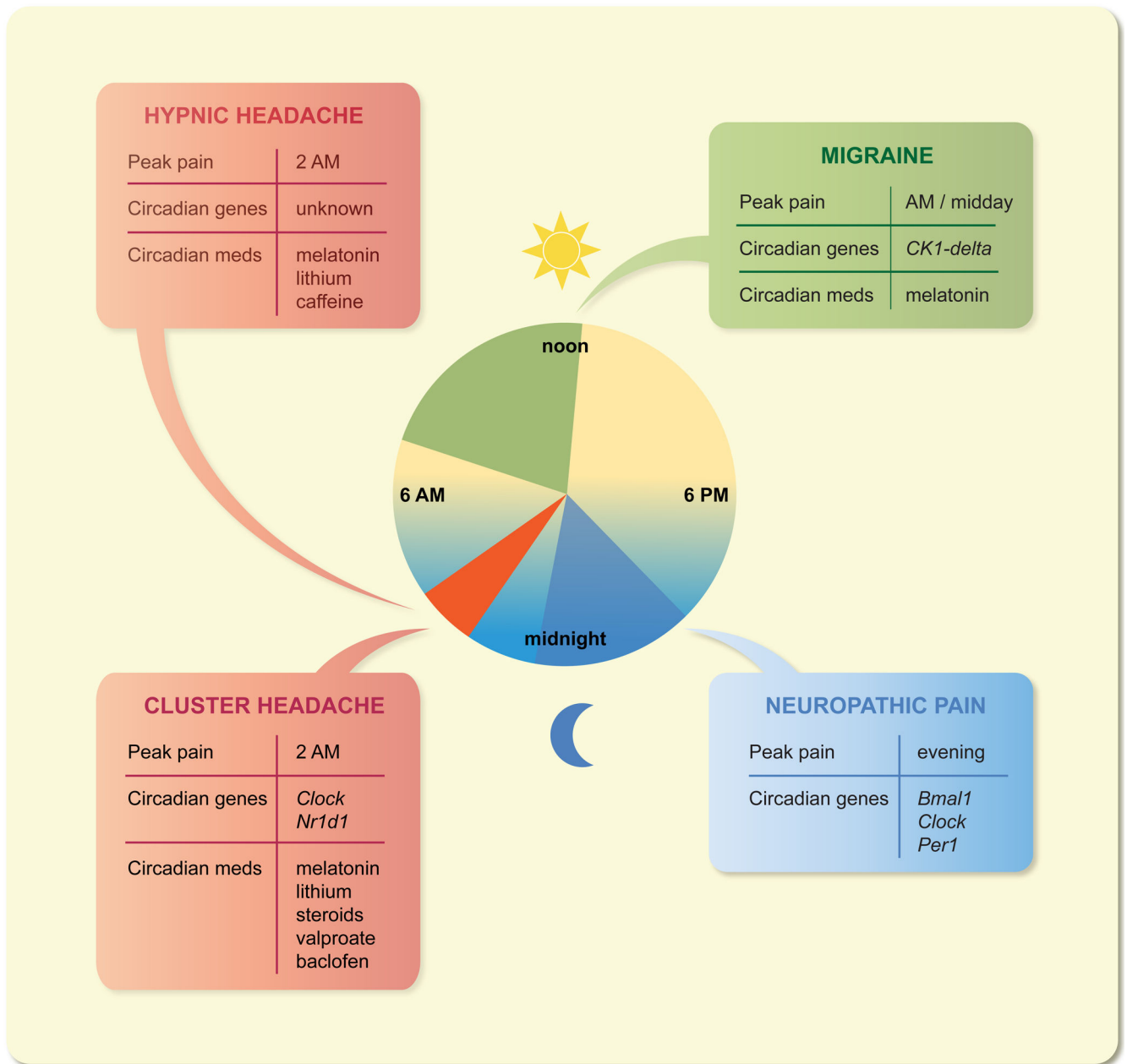
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**Figure 1:**

The cell-autonomous circadian oscillator present in brain and peripheral cells. In the oscillator, transcriptional-translational feedback loops are connected and reciprocally regulated, including the core loop (CLOCK/NPAS2, BMAL1 and PERs, CRYs) and secondary loop (BMAL1, REV-ERBs and RORs). The oscillators are fine-tuned by a wide array of regulatory factors (including the CK1 and GSK3 $\beta$  kinases) and input signals and in turn drive expression of output genes in a tissue-specific manner, thus controlling metabolic, physiological, behavioral and cognitive functions. Full name of genes encoding the clock components are as follows. *Clock*: Circadian Locomotor Output Cycles Kaput; *Npas2*:

Neuronal PAS domain protein 2; *Bmal1*: Brain And Muscle ARNT-Like 1; *Per*: Period; *Cry*: Cryptochrome; *Rev-erb*: Reverse strand of erba; *Ror*: Retinoid acid-related Orphan Receptor; *Ckl*: casein kinase 1; *Gsk3b*: glycogen synthase kinase 3 beta. The genes encoding the nuclear receptors RORs and REV-ERBs are also known as *Nr1f* and *Nr1d* subfamilies, respectively. Modified from<sup>137</sup> under Creative Commons Attribution license.



**Figure 2:** Circadian rhythms in headaches and neuropathic pain. Headache disorders, including cluster headache, migraine and hypnic headache, and neuropathic pain display circadian rhythmicity of attack occurrence. Several clock components have also been implicated in these diseases in either rodent models or in small human studies. Note that *Nr1d1* encodes REV-ERB $\alpha$ . Several pain medicines, such as lithium, valproic acid and melatonin and corticoids, are known to reset the circadian clock.