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Review

The contribution of clock genes BMAL1 and PER2 in osteoarthritis-associated pain☆

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ABSTRACT

Joint pain is the primary symptom of osteoarthritis (OA) and the main motivator for patients to seek medical care. OA-related pain significantly restricts joint function and diminishes quality of life. Despite the availability of various pain-relieving medications for OA, current treatment strategies often fall short in delivering adequate pain relief. Furthermore, long-term use of pain medications for OA management is frequently linked with notable side effects and toxicities, suggesting the need to explore new potential targets to treat pain in OA patients. In this context, clock genes, particularly brain and muscle aryl hydrocarbon receptor nuclear translocator-like 1 (BMAL1) and period circadian protein homolog 2 (PER2), known for their role in circadian rhythms, represent promising opportunities for pharmacological interventions due to their involvement in both the development and maintenance of OA pain. While BMAL1 and PER2 have been extensively studied in neuropathic and inflammatory pain, their specific contributions to OA pain remain less clear, demanding further investigation. This narrative review aims to synthesize the relationship between OA pain and the BMAL1 and PER2 signaling pathways, ultimately exploring the potential therapeutic role of clock genes in addressing this challenging condition.

1. Introduction

Osteoarthritis (OA) is the most prevalent chronic pain-evoking and disability-causing joint disease, affecting approximately 32 million adults in the USA (Hunter & Bierma-Zeinstra, 2019). Despite its high prevalence, the economic burden of OA, including healthcare costs and lost productivity, is estimated at $165 billion (Katz et al., 2021). Notably, OA is commonly associated with comorbidities such as physical inactivity, depression, anxiety, and metabolic syndromes such as obesity, diabetes and heart disease (Katz et al., 2021). OA affects multiple joint structures including the synovial membrane, subchondral bone, menisci, infrapatellar fat pads, and cartilage (Chen et al., 2017). The pathophysiology of OA includes cartilage fibrillation and loss, subchondral bone remodeling, and chronic, low-grade inflammation across the entire joint, which ultimately compromise joint stability, functionality, and precipitates pain (Chen et al., 2017; Lu et al., 2022). OA pain is a multifaceted condition influenced by genetic, psychological, and environmental factors. Unlike the structural changes seen in OA, the related pain is a subjective experience arising from both peripheral and central neural mechanisms. Several studies have examined the complex nature of OA pain, investigating peripheral sensitization, central pain pathways, immune responses, and its structural contributors (Eitner et al., 2017). It is clear that a deeper understanding of these mechanisms is essential, as it could lead to more effective, mechanism-based treatments for OA pain. From the patient's perspective, it begins as movement-evoked or associated with activity, progresses to an unpredictable and intermittent dull ache, and then becomes more frequent and debilitating as the disease advances. In the early stages, most

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patients self-manage their pain with NSAIDs, causing the pain to go unnoticed clinically until it becomes more unpredictable and challenging to manage (Song et al., 2018). Despite this, current pharmacological strategies for OA pain treatment are limited and associated with severe side effects and addiction risk, failing to provide satisfactory relief (Mezey et al., 2022; Nelson et al., 2014).

An emerging area of interest in OA research is the role of circadian rhythms, where the disruption and alterations in clock genes have been reported to contribute to the pathogenesis of OA (Dudek et al., 2016; Gossan et al., 2013). In this context, increasing evidence suggest that clock genes may play a crucial role in OA-induced pain (Bumgarner et al., 2021; Das et al., 2018; Segal et al., 2018). In this context, preliminary results from our group suggest that a hypomethylation in a probe located at an exon of the period circadian protein homolog 3 (PER3) gene is associated high impact chronic pain (Montesino-Goicolea et al., 2024). Moreover, our own ongoing work is focused in understanding differential DNA methylation in brain and muscle aryl hydrocarbon receptor nuclear translocator-like 1 (BMAL1) and PER2 in individuals with knee OA. These insights have driven efforts to develop innovative therapies targeting specific clock genes such as BMAL1 and PER2, using specific antagonists to mitigate inflammation, cartilage degeneration, pain, and other pathways, ultimately alleviating OA symptoms. Therefore, this review aims to provide insights into OA, focusing on the emerging roles of clock genes in the development and persistence of OA-induced pain. It also explores the underlying mechanisms of OA and the interaction between circadian rhythms and pain threshold fluctuations.

2. Pain mechanisms implicated in osteoarthritis

Pain is one of the most prevalent symptoms in patients with OA, being strongly associated with reduced function across various aspects of daily life. The surrounding tissues of the joint (i.e., synovium, fat pad, ligaments, joint capsule, and subchondral bone), are densely innervated by the sympathetic and sensory nerves, enabling proprioception, vasoregulation, and nociception (Hines et al., 1996; McDougall, 2006). Nociceptive fibers, specially Aδ and C fibers, widely innervate structures such as synovial capsule, ligaments, menisci, and subchondral bone, responding to mechanical or chemical stimuli linked to OA pain (Mease et al., 2011). Evidence suggests that the OA pain is largely mediated by nociceptive mechanisms rather than structural damage alone. For instance, intra-articular bupivacaine injection reduces knee OA pain, highlighting that activation of nociceptive pathways in the joint drives OA pain (Creamer et al., 1996). Notably, OA induces the upregulation of voltage-gated sodium channels Nav1.8 and Nav1.7 in afferent neurons that innervate the swollen knee (Hestehave et al., 2024; Schuelert & McDougall, 2012), suggesting that these channels are also implicated in the noxious sensing mechanisms in OA. In support of this, our findings show that blocking SUMOylation-dependent Nav1.7 trafficking reduces its functional activity, producing an antinociceptive effect in the monoiodoacetate model of OA-like pain (Hestehave et al., 2024). Beyond its role in afferent neurons, Nav1.7 has been identified as a novel OA-associated channel expressed in human chondrocytes (Fu et al., 2024). Functional Nav1.7 channels in chondrocytes regulate intracellular calcium signaling and influence the chondrocyte secretome, contributing to OA progression and joint damage. Inhibition of Nav1.7 or NCX1 (sodium/calcium exchanger 1) in chondrocytes not only reduced the release of HSP70, which sensitizes nociceptors and exacerbates OA pain, but also significantly ameliorated structural joint damage and pain behaviors in OA models (Fu et al., 2024). These findings highlight Nav1.7 as a dual therapeutic target, addressing both the nociceptive and structural components of OA pathophysiology (Fu et al., 2024). In addition to this, compressive forces and locomotion may trigger the activation of mechano-gated ion channels such as Piezo channels on sensory terminals that innervate the joints (Heppelmann & McDougall, 2005). Deletion of Piezo2 from nociceptors prevents mechanical sensitization in experimental OA (Obeidat et al., 2023), suggesting a potential role in OA pain. Conversely, Piezo 1 activation in chondrocytes promotes degenerative processes in the joint, through the upregulation of matrix metalloproteinases (MMPs) (Gao et al., 2022). However, whether Piezo1 has a direct role in OA-related pain is currently unknonwn. Additionally, repeated high impact loading, obesity, or aging can reduce the activation threshold of these channels, contributing to pain (Gwilym et al., 2009; Hochman et al., 2010; McDougall et al., 2009).

In addition to Nav and Piezo channels, transient receptor potential (TRP) channels, specifically TRPV1 and TRPV4, play an important role in the OA pain and inflammation. Notably, the TRPV1 channel acts as a transducer of chemical and inflammatory pain signals in chondrocytes (Chen et al., 2024), while the upregulation of TRPV4 is linked to cartilage extracellular matrix degradation, synovial inflammatory response, and hyperalgesia (Xing et al., 2017). Altogether, these findings suggest that TRP channels contribute to both anti-inflammatory and analgesic processes within the joint.

Although OA has historically been labeled as a “non-inflammatory” arthritis, low-grade upregulation of inflammatory mediators is now widely accepted to play a key role in OA pain. Nociceptors in the joint express a broad range of receptors for inflammatory molecules, such as cytokines, chemokines, neuropeptides, and prostaglandins, which contribute to peripheral sensitization and pain in OA (Grace et al., 2011). Indeed, the presence of proinflammatory cytokines such as interleukin-1β (IL-1β), interleukin-6 (IL-6), or tumor necrosis factor alpha-(TNF-α) active nociceptors in OA joints, leading to persistent sensitization and increased pain in affected patients (Brenn et al., 2007; Richter et al., 2010).

On the other hand, central sensitization, resulting from tonic nociceptive input from the injured joint, leads to increased excitability and a lowered of the excitation threshold of spinal cord neurons, thereby amplifying pain signals. In OA animal models, this process is associated with elevated levels of substance P, calcitonin gene-related peptide (CGRP), glutamate and bradykinin, all contributing to spinal hyperexcitability (Ferland et al., 2011; Inglis et al., 2007; Puttfarcken et al., 2010). Additionally, the release of nociceptor-sensitizing mediators like nerve growth factor (NGF) during cartilage degradation, bone remodeling, or the synovial inflammation further contributes to sensitization by lowering the activation threshold of nociceptors, thus amplifying pain response (Wood et al., 2022). Notably, blocking NGF has been shown to reduce OA-induced pain (Shelton et al., 2005), highlighting its role in sensitizing nociceptors in OA. Furthermore, the development of pain in OA models is linked to an increase of microglia in the spinal cord (Eitner et al., 2017), suggesting a neuroinflammatory component that plays a role in central sensitization. However, central sensitization mechanisms during the OA process are complex and have not been extensively studied.

In summary, the complexity of OA pain arises from the interactions among various joint cells and sensory neurons, involving components such as proinflammatory cytokines, mechano-gated ion channels, growth factors, neuropeptides, and glial cells (Fig. 1). Although the mechanisms underlying nociceptive signaling in OA are extensive and not yet fully understood, this review explores the potential implications of chronobiology in OA pain. Based on our ongoing research in human knee OA patients, we focus on the roles of BMAL1 and PER2 as a potential research arc for OA-induced pain. Therefore, this review explores the involvement of BMAL1 and PER2 in OA pain to address this critical gap and inform our understanding of OA, laying the basis for therapeutic interventions that could significantly enhance patient outcomes.

3. Clock genes

The circadian clock is a self-sustaining 24-hour oscillator that controls a wide range of activities including, blood pressure, body temperature, and hormone secretion, among others (Dunlap, 1999). Circadian

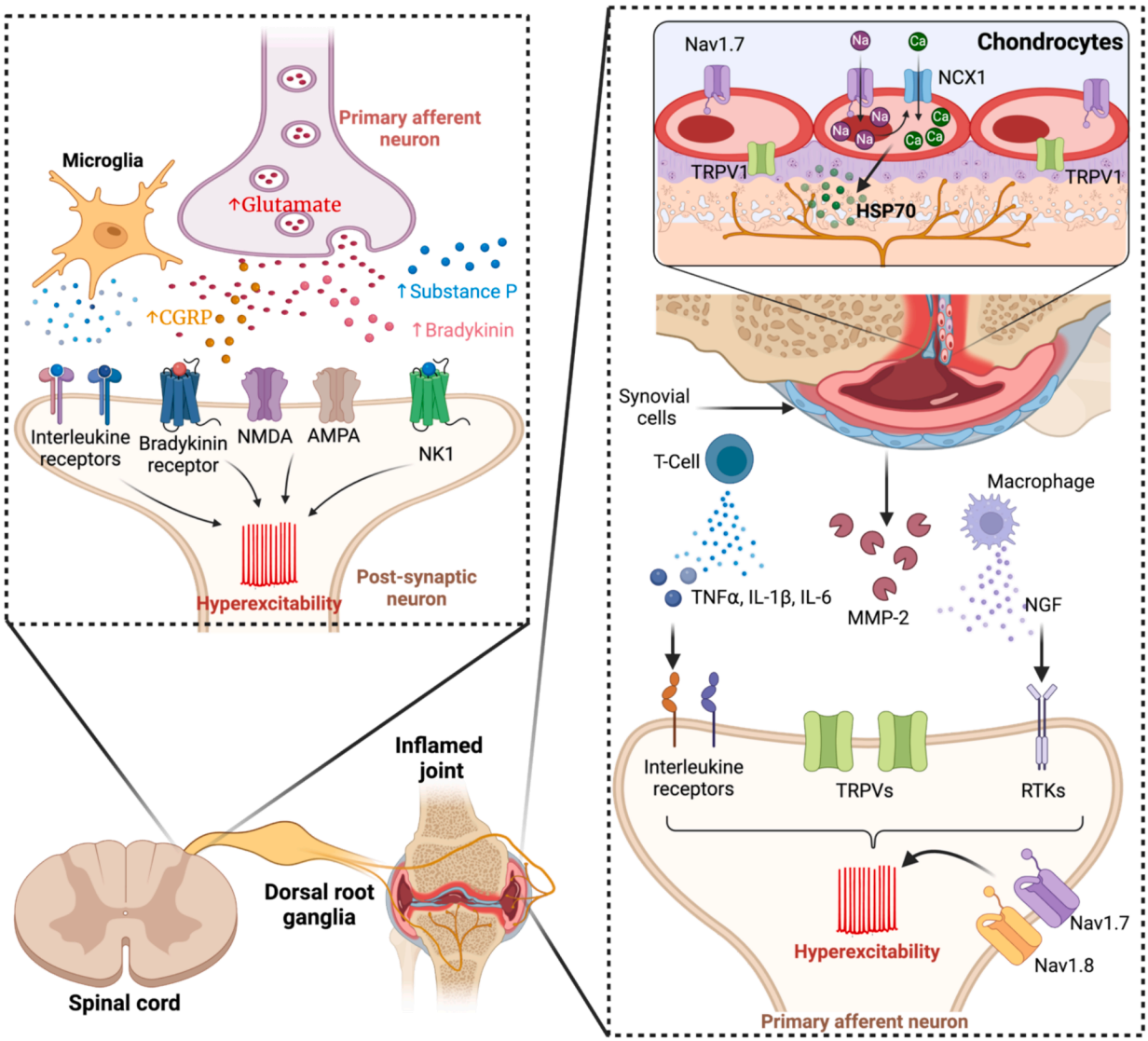

Fig. 1. Pathophysiological mechanisms implicated in osteoarthritis. OA pain involves several complex pathophysiological mechanisms that contribute to increased neuronal activity of sensory fiber innervating the joint, leading to central sensitization within the spinal cord. Upregulation of voltage-gated sodium channels (i.e., Nav1.7 and Nav1.8), tyrosine kinase receptors, and transient receptor potential cation channel subfamily V (TRPV) contributes to increased neuronal excitability. Additionally, infiltration of immune cells (macrophages) proximal to nerve endings promotes an inflammatory environment. The up-regulation of pro-nociceptive molecules such as TNF-α, IL-1β, and IL-6 released from immune cells further enhances pain. Chondrocytes express ions channels that indirectly contribute to pain signaling. Elevated intracellular calcium concentrations resulting from of the activation of TRPV1 or sodium calcium exchange 1 (NCX1) channels in chondrocytes has been linked to the release of heat shock protein 70 (HSP70), which can activate nociceptors localized around the joint. At the spinal cord level, elevated levels of substance P, bradykinin, CGRP and glutamate enhance the activity of NK1, bradykinin receptors, NMDA and AMPA receptors, thereby increasing neuronal excitability. Moreover, microglia cells release pro-inflammatory cytokines into the spinal dorsal horn, promoting the establishment of central sensitization. Abbreviations are as follows: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; CGRP, calcitonin gene-related peptide; IL-1β, interleukin-1 beta; IL-6, interleukin-6; MMP-2, matrix metalloproteinase-2; Nav1.7, voltage-gated sodium channel subtype 1.7; Nav1.8, voltage-gated sodium channel subtype 1.8; NCX1, sodium/calcium exchanger 1; NGF, nerve growth factor; NK1, neurokinin 1 receptor; NMDA, N-methyl-D-aspartate receptor; RTKs, receptor tyrosine kinases; TNF-α, tumor necrosis factor alpha; TRPV1, transient receptor potential vanilloid-1.

regulation offers evolutionary advantages, increasing the energy efficiency of physiological processes such as body temperature control, wakeup/sleep and food intake, and allowing organisms to predict rhythmic environments and diurnal changes (Dunlap, 1999; Hastings, 1997). In mammals, the suprachiasmatic nucleus (SCN), located in the medial anterior hypothalamus, acts as the master synchronizer of circadian rhythms (Moore & Lenn, 1972). The SCN receives external light signals through the retinohypothalamic tract, which transmits information form light-sensitive neurons in the retina (Guler et al., 2008). This pathway plays an important role in associating the SCN to the light cycle, alongside other signals like temperature and feeding patterns, thereby maintaining the body's 24-hour rhythm (Man et al., 2016). Through its influence on the hypothalamic–pituitary–adrenal (HPA) axis, the SCN regulates rhythmic glucocorticoid release, which serves as a systemic signal to align peripheral clocks across tissues and cells, ensuring coordinated physiological processes for optimal functioning (Damiola et al., 2000; Man et al., 2016).

At the molecular level, the regulation of circadian rhythms relies on "clock genes" present in every cell type. These genes form transcriptional-translational autoregulatory feedback loops, which are cell autonomous and highly conserved in both the SCN and peripheral cells (Asher & Schibler, 2011). The core clock genes consist of four main genes: circadian locomotor output cycles kaput (CLOCK), BMAL1 (also known as ARNTL), period genes (PER1/2/3), and cryptochrome genes

(CRY1/2) (Asher & Schibler, 2011; Brown, 2016). During the morning, CLOCK and BMAL1 form a complex and bind to E-box response elements in the promoter regions of the repressor clock genes, PER1/2/3 and CRY1/2 (Asher & Schibler, 2011). Throughout the day, PER and CRY levels increase, peaking in the evening. At this peak, they move into the nucleus to inhibit the activity of the CLOCK/BMAL1 complex. Since clock genes are implicated in the regulation of up to 15% of genes within a given tissue, desynchronization of circadian rhythms has been linked to the pathogenesis of numerous disease conditions (Panda et al., 2002; Takahashi, 2017). Indeed, circadian rhythms play a role in regulating various physiological and pathological processes, including the perception and experience of pain (Bruguerolle & Labrecque, 2007; Bumgarner et al., 2021). Thus, the complex relationship between circadian rhythms and pain modulation has gained significant attention within the field of pain, providing insights into how pain fluctuates throughout the day and night.

4. Chronobiology of pain

4.1. Circadian variation of pain

It has been reported that pain sensitivity and perception exhibit daily fluctuations that align with circadian rhythms (Bumgarner et al., 2021; Palada et al., 2020; Segal et al., 2018). Although the precise mechanisms behind circadian regulation in pain are not fully understood, both clinical and preclinical evidence indicate that pain is regulated by the circadian system (Bumgarner et al., 2021; Mun et al., 2022). Early preclinical studies revealed that nociceptive behaviors in hamster (Pickard, 1987) and mice (Oliverio et al., 1982) followed rhythmic patterns. Notably, variations in pain responsiveness observed in these studies were obtained from rodents kept under constant conditions, suggesting that these fluctuations represent true circadian rhythms rather than being solely influenced by environmental factors (Oliverio et al., 1982; Pickard, 1987). In this context, it has been shown that mechanical and thermal withdrawal thresholds of the rodent paws are lower in the morning compared to noon (Frederickson et al., 1977; Piña-Leyva et al., 2022; Wesche & Frederickson, 1979), suggesting circadian rhythms in mechanosensitivity and thermosensitivity. In contrast, some studies have reported that pain sensitivity peaks during the active phase (Frederickson et al., 1977; Martínez-Gómez et al., 1994; Oliverio et al., 1982). The discrepancies may be due to differences in the pain assessment paradigms used or the influence of other factors such as sleep or vigilance (Mun et al., 2022).

At the same time, preclinical findings are supported by some clinical studies reporting that healthy subjects exhibit increased sensitivity to electric and radiant heat stimuli in the mornings (Davis et al., 1978; Martin et al., 1914; Procacci et al., 1974). Additionally, a study showed that mechanical stimuli applied to the forehead using an inflatable cuff or tourniquet were associated with a nighttime peak in sensitivity (Gobel & Cordes, 1990; Koch & Raschka, 2004). These circadian variations in pain sensitivity are influenced by factors such as changes in body temperature, hormonal fluctuations, and neural activity patterns (Knezevic et al., 2023). Indeed, a mathematical model exploring whether pain sensitivity follows a daily rhythm suggested that, in the early afternoon (4–8 h after waking), there is an increase in pain inhibition because the Aβ-fibers are more sensitive to external stimuli compared to C-fibers. In contrast, in the middle of the night (16–20 h after waking), pain is higher compared to the afternoon, suggesting that physiological pain inhibition is less effective during these times (Crodelle et al., 2019). However, we acknowledge that many preclinical data about circadian fluctuations in pain have been obtained by using rodent animal models. Since rodents are nocturnal animals, these rhythms may be reverse in a nocturnal setting, potentially limiting the direct translation to humans.

A similar phenomenon is observed during pathological conditions, where several chronic pain disorders exhibit circadian fluctuations in pain intensity. For example, mice with neuropathic pain-like behaviors experience less mechanical allodynia in the mornings (8:00 am), with pain intensity increasing during the day (14:00 – 20:00 pm) and decreasing again in the early morning (2:00 am), while thermal pain remains unchanged over a 24-h period, suggesting circadian variations in pain sensitivity under neuropathic pain conditions (Takada et al., 2013). Notably, clinical studies have reported that neuropathic pain tends to worsen in patients during the night (Belgrade, 1999; Galer et al., 2000). Moreover, the pain intensity of patients with diabetic neuropathy and post-herpetic neuralgia tends to increase during the day (Gilron et al., 2013; Odrcich et al., 2006), while some studies have found that diabetic neuropathy, postherpetic neuralgia and multiple sclerosis present with pain peak at night (Bumgarner et al., 2021). Conversely, morning pain peaks are more common in patients with migraine, cancer, and fibromyalgia (Bellamy et al., 2004; Kowanko et al., 1981; Levi et al., 1985; Solomon, 1992). Similarly, patients with rheumatoid arthritis and OA demonstrate unique circadian patterns in their pain severity. For instance, patients with rheumatoid arthritis often report higher pain sensitivity in the morning (Cutolo & Masi, 2005; Kowanko et al., 1981), whereas OA pain severity tends to peak in the evening hours (Bellamy et al., 1990; Levi et al., 1985). This discrepancy in circadian pain patterns may be attributed to extrinsic factors such as variation in study subjects or the etiologies of these diseases. Additionally, intrinsic factors, such as clock genes, contribute to changes in the expression of substances or receptors implicated in pain signaling, thus influencing pain circadian rhythms (Knezevic et al., 2023). Therefore, exploring the role of clock genes during chronic pain conditions, particularly focusing on osteoarthritis, emerges as a promising avenue for treatments that could significantly impact patient outcomes.

4.2. Sleep and pain

Pain sensitivity is influenced by both sleep-related and circadian mechanisms, which often overlap, making it challenging to clearly disentangle their individual contributions. Sleep disturbances, such as deprivation or fragmentation, are well-established factors that enhance pain perception and exacerbate chronic pain conditions, including low back pain, neuropathic pain, and fibromyalgia (Zhu & Huang, 2023). These effects are partly mediated by proinflammatory pathways (Dimitrov et al., 2015; Haack et al., 2007; Hu et al., 2003; Irwin et al., 2015; Manchanda et al., 2018; Rodríguez-Palma et al., 2024), and central sensitization (Nijs et al., 2018).

Previous reports using experimental models of sleep deprivation have demonstrated that restricted or disruption of sleep cycles over consecutive days leads to hyperalgesia and can trigger spontaneous pain (Roehrs et al., 2006; Smith et al., 2007). However, more recent study highlighted a potentially more prominent role for circadian rhythms in modulating sensory and pain thresholds (Daguet et al., 2022). In that study, the authors demonstrated that pain sensitivity exhibits a strong circadian rhythm, peaking during the night and reaching its lowest levels in the afternoon. Their findings suggest that circadian mechanisms account for the majority of fluctuations in pain sensitivity, whereas sleep-related processes exert a more modest influence (Daguet et al., 2022). This rhythmic modulation of pain emphasizes the importance of considering circadian factors in the assessment and management of chronic pain. Moreover, the relationship between sleep deficiency and pain creates a reinforcing cycle that contributes to the shaping of pain perception, where poor sleep increases pain sensitivity, which further disrupts sleep, resulting in an ongoing and intensifying pattern over time. Therefore, this dynamic interplay between circadian rhythms and sleep disturbances highlights the need for a more integrated approach to chronic pain research, which accounts for both circadian biology and sleep health in the development of effective management strategies.

4.3. Transcriptomic regulation of clock genes in pain conditions

As previously discussed, the relationship between circadian rhythms and pain perception is emerging as an important factor in understanding chronic pain conditions. Clock genes, specially BMAL1 and PER2, are essential for regulating these rhythms and have also been linked to the modulation of pain sensitivity. Advances in transcriptomic techniques, particularly with single nucleus RNA sequencing (snRNA-seq) and single-cell RNA sequencing (scRNA-seq), have enhanced our understanding of molecular mechanisms underlying chronic pain conditions in both rodents and humans (Bangash et al., 2018; Chu et al., 2023; Kim et al., 2020; Ray et al., 2019). A single-nucleus transcriptome analysis of human and mouse trigeminal ganglion (TG) showed that certain core clock genes such as ARNTL (BMAL1), CLOCK, CRY2, NR1D2, PER1, PER3 and TEF are expressed at similar levels in both species (Chu et al., 2023). Conversely, species-specific differences were observed in core clock genes, such as CRY1, PER2 and NR1D1. CRY1, a negative feedback regulator of the molecular clock that inhibits ARNTL/CLOCK heterodimers (Albrecht, 2012), is more expressed in human TG compared to mouse TG. In mice, CRY1 levels are slightly elevated in neurons (15.6 %) compared to other cell types. Additionally, PER2 is expressed in a limited range of human TG cells like neurons, fibroblasts, and endothelial cells, but it is expressed in more cell types within the mouse TG. Moreover, NR1D1, which suppresses ARTNL/BMAL1 expression, has low expression levels across various cell types in the human TG but is more expressed in mouse neurons (11.3 %) (Chu et al., 2023). It is important to note that these studies analyzed clock gene expression at a single time point across different cell types rather than over multiple circadian phases. This single-point approach limits the ability to capture the dynamic fluctuations typically observed in circadian gene expression. As a result, the differences observed may represent baseline variations rather than true circadian oscillations. This limitation highlights the need for further research using time-course data to fully understand interspecies differences in circadian regulation, especially for pain-related clock genes in humans and mice.

An analysis of 248 human tibial nerve transcriptomes from donors with arthritis or rheumatoid arthritis revealed that ARNTL, PER1, PER2, PER3, CLOCK, CRY1, CRY2, and *RORA* are expressed in human sensory neurons (Ray et al., 2019). Additionally, microarray data suggest that OA pain induced by the surgical destabilization of medial meniscus develops in distinct stages, with each stage showing unique molecular expression patterns in sensory neurons from DRGs. Circadian-related genes such as ARNTL, PER3, and NR1D1 are upregulated during both early and late phases of OA pain, while genes like PER1, PER2, CLOCK, CRY2 are upregulated only in the late phase (Miller et al., 2020). This differential expression of clock genes suggest that different phases of OA pain may involve distinct molecular pathways, and different roles for clock genes. In contrast, a study using the mechanical joint loading model to induce OA pain reported no significant differential expression of circadian genes such as ARNTL, CLOCK, PER1, PER3, RORA and NR1D1 in sensory neurons from DRGs (Bangash et al., 2018). This discrepancy indicates that the relationship between circadian genes and OA pain may vary depending on the specific pain model used. Consequently, findings from different models may not always align, emphasizing the need to harmonize transcriptomic data with functional studies across various OA models. Further research should focus on validating these molecular changes in different OA models and correlating them with nociceptive outcomes to ensure a more comprehensive understanding of the circadian regulation of OA pain.

5. Role of clock genes in pain modulation

Several lines of evidence indicate that clock genes are implicated in various chronic pain conditions such as migraine, chemotherapy-induced pain, post-surgical pain, rheumatoid arthritis and OA pain (Bumgarner et al., 2021; Segal et al., 2018). Genes involved in the pathophysiology of pain have been reported to follow a circadian rhythm and are regulated by clock genes (Das et al., 2018; Kim et al., 2020). For instance, a transcriptomic study found that genes encoding for Na_V, K_V, and TRP channels display a diurnal expression pattern in DRG and spinal cord in a model of chemotherapy-induced neuropathy (Kim et al., 2020). Additionally, both the protein and mRNA levels of α2δ-1, an auxiliary subunit of voltage-gated calcium channels, increases and fluctuates in a time-dependent pattern in DRG sensory neurons following nerve injury (Kusunose et al., 2010). Altogether, these findings suggests that the expression of pain-related transcripts, proteins, and ion channels activity may vary across the day, highlighting the potential role of clock genes in circadian pain perception and modulation. However, the lack of studies in this area represents a significant gap in our understanding, leaving unanswered a reservoir of potential insights that could transform the way we approach and treat OA pain. Thus, in the following sections, we will summarize the current evidence supporting the role of clock genes in OA pain, with a focus on how clock genes may contribute to the circadian rhythms during OA-pain. We will also explore how disruptions in specific clock genes such as BMAL1, PER2, and other genes such as CRY1/2 and CLOCK in sensory neurons, immune cells or chondrocytes can contribute to enhance pain signaling pathways during OA-related pain Table 1 (Fig. 2).

5.1. BMAL1 as a novel target for OA-induced pain

BMAL1 is expressed in sensory neurons and satellite glial cells of the dorsal root ganglia (DRG) and in the spinal cord (Das et al., 2018; Kim et al., 2020; Zhang et al., 2012). It regulates the expression of substance P, a neuropeptide implicated in pain signaling, in the DRG but not in the spinal cord (Zhang et al., 2012). In support of this, BMAL1 deletion leads to downregulation of key pain-related proteins, such as substance P, CGRP, TrkA, TRPV1, $Na_v1.8$ and $Na_v1.7$ in nociceptors, suggesting that BMAL1 plays a pronociceptive role in pain conditions (Das et al., 2018). Notably, in an OA model induced by partial medial meniscectomy (PMM) of the knee, BMAL1 expression was upregulated in DRG neurons, while the deletion of BMAL1 from $Na_v1.8^+$ sensory neurons prevented the development of mechanical hyperalgesia. This effect was linked to reduced expression of TRPV1, CGRP, TrkA, substance P and NGF in BMAL1 KO mice—all key players in pain signaling. Furthermore, pharmacological activation of REV-ERB (a transcriptional factor that negatively regulates BMAL1 activity) using SR9009 reduced BMAL1 levels and diminished OA-induced pain (Das et al., 2018; Hashizume et al., 2024). Interestingly, SR9009 treatment also decreased cartilage destruction, despite the protective role of BMAL1 in cartilage. Of note, SR9009 has effects on cellular survival and metabolism independently of REV-ERB activation, which may suggest that its beneficial effects in cartilage are clock-independent (Dierickx et al., 2019). Importantly, OA pain is closely linked to the degree of cartilage damage with worsening chondrocyte metabolism leading to increased pain intensity (Nwosu et al., 2016). Since BMAL1 is also expressed in chondrocytes, it represents a promising target for managing OA pain not only through its regulation of ion channels and proinflammatory molecules involved in pain signaling, but also due to its protective role in cartilage integrity.

Transcriptional repression of BMAL1 has been associated with reductions in inflammatory molecules such as matrix metalloproteinases (e.g., MMP3, MMP9, MMP13) and cytokines like interleukin-1β and tumor necrosis factor in lipopolysaccharide-treated primary cultured chondrocytes (Hashizume et al., 2024). BMAL1 also binds to promoter regions of immune genes such as TLR-9, CCL-2, and CCL-8 in monocytes (Nguyen et al., 2013). The circadian production of these chemokines by monocytes at sites of inflammation can directly activate or sensitize joint nociceptors, leading to neuronal hyperactivity and contributing to OA pain (Segal et al., 2018). Inhibition of BMAL1 results in decreased NF-kB activation and downregulation of proinflammatory cytokines (Narasimamurthy et al., 2012), further suggesting its role in chronic pain modulation.

Table 1
Preclinical studies on the role of clock genes in chronic pain conditions.

Gene	Model	Species	Main results	Conclusion	Reference
Bmal1	NA	Male C57BL6/J mice	• BMAL1 exhibits circadian expression in DRG neurons. BMAL1 regulates the circadian expression of *Tac1* gene (substance P) in DRG neurons.	BMAL1 is involved in the transcriptional regulation of pro-nociceptive molecules (substance P).	(Zhang et al., 2012)
Bmal1	Partial medial meniscectomy of the knee	Female C57BL6/J mice	• BMAL1 expression is upregulated in DRG neurons. SR9009 injection reduces BMAL1 expression and decreases mechanical hyperalgesia. BMAL1 deletion in Nav1.8^{+} neurons reduces mechanical hyperalgesia.	Disruption of the molecular clock, through genetic deletion of BMAL1 in sensory neurons, reduces OA pain.	(Das et al., 2018)
Bmal1	Monosodium iodoacetate-induced osteoarthritis	Male DDY mice	• SR9009, REV-ERB agonist, prevented upregulation of cytokines in chondrocytes. Intraarticular SR9009 treatment reduces MIA-induced mechanical hypersensitivity. Intraarticular SR9009 treatment partially inhibits MIA-induced cartilage degradation.	Transcriptional repression of BMAL1 using SR9009 alleviates OA pain by negatively regulating inflammation in chondrocytes.	(Hashizume et al., 2024)
Clock	NA	*Clock*$^{\Delta 19}$ mutant mice	• Clock mutation promotes cartilage degradation and up-regulates IL-1β, IL-6 and MCP-1. Exogenous Clock expression inhibits NFκB activation, reducing cartilage damage and inflammation.	Disruption of Clock increases the risk of developing osteoarthritis.	(Yuan et al., 2019)
Cry1/2	Collagen antibody-induced arthritis	Male DBA/1J mice	• Melatonin reduces *CRY1/2* levels. Melatonin injection exacerbates CIA-induced paw inflammation. Melatonin administration increases type II collagen, and *TNF*-α levels.	Attenuation of *Cry1* by melatonin may contribute to the aggravation of anti-type II collagen antibody-induced arthritis.	(Bang et al., 2012)
Cry2	Destabilization of the medial meniscus	Male and Female Cry2 global deletion mice	• *Cry2* expression is downregulated in mice with aging-related OA. *Cry2* KO increases cartilage damage.	*CRY2* is a circadian rhythm gene essential for cartilage homeostasis during OA.	(Bekki et al., 2020)
Per1	Partial sciatic nerve ligation	Male CD-1 mice	• *Per1* expression decreases in the spinal cord after nerve injury. Reduced *Per1* expression enhances JNK phosphorylation in the spinal cord. *Per1* KO increases JNK phosphorylation, boosts CCL2 production and induces allodynia.	Downregulation of *Per1* in the spinal dorsal horn plays a role in the induction of neuropathic pain.	(Morioka et al., 2016)
Per2	Restrain movement-induced stress	*Male Per2* mutant mice	• Grooming bouts and durations remain unaffected in *Per2* mutant mice. *Per2* mutant mice display significantly higher latency in the hot plate test following stress exposure. *Per2* mutant mice exhibit increased mechanical thresholds after stress exposure.	*Per2* may play an important role in the mechanical and thermal antinociception observed after acute stress exposure.	(Zhang et al., 2011)
Per2	Opioid-induced hyperalgesia	Male C57BL6/J mice	• OIH induces upregulates *Per2* expression in the NAcc.	*Per2* may be implicated in the opioid-induced hyperalgesia.	(Zhang et al., 2019)
Per2	NA	*Per2 KO mice*	• *Per2* deletion enhances morphine-induced analgesia. *Per2* deletion increases conditioned-place preference to morphine. *Per2* deletion upregulates μ-opioid receptor expression in the NAcc.	*Per2* influences opioid-mediated pain relief and reward mechanisms.	(Custodio et al., 2022)
Per2	Partial sciatic nerve ligation	Male *Per2*$^{m/mm}$ mice	• *Per2*$^{m/m}$ mice fail to develop tactile hypersensitivity after PSL. *Per2*$^{m/m}$ mice show activation of glial cells in the spinal cord. α_{1D}-adrenergic receptor expression is up-regulated in the spinal cord of *Per2*$^{m/m}$ mice.	*The Per2* gene prevents PSL-induced pain by increasing α_{1D}-AR expression in the spinal cord, which promotes the production of 2-AG.	(Yamakawa et al., 2024)

2-AG, 2-Arachidonoylglycerol; *Bmal1*, Brain and muscle arnt-like 1; CCL2, C-C motif ligand 2; CIA, Collagen antibody-induced arthritis; *Clock*, Circadian locomotor output cycles kaput; *Cry 1/2*, Cryptochrome 1/2; DRG, Dorsal root ganglia; IL-1β, Interleukin 1 beta; IL-6, Interleukin 6; JNK, c-Jun N-terminal kinase; KO, Knockout; MCP-1, Monocyte Chemoattractant Protein-1; MIA, Monoiodoacetate; NA, Not applicable; NAcc, Nucleus accumbens; NFkB, Nuclear factor kappa B; OA, Osteoarthritis; OIH, Opioid-induced hyperalgesia; *Per1*, Period 1; *Per2*, Period 2; PSL, Partial sciatic nerve ligation; REV-ERB, Reverse c-erbAα; TNF-α, Tumor necrosis factor-alpha.

Beyond OA, BMAL1 has been also reported in others pain conditions. For instance, paclitaxel-induced neuropathic pain exhibits a circadian rhythm in tactile allodynia, linked to the upregulation of BMAL1 in the DRG (Kim et al., 2020). Additionally, *BMAL1* modulates inflammatory rhythmic pain by regulating the oscillatory expression of substance P in the DRG (Zhang et al., 2012). Collectively, these findings highlight the potential of BMAL1 as a therapeutic target for modulating circadian mechanisms underlaying pain fluctuations in OA and other chronic pain

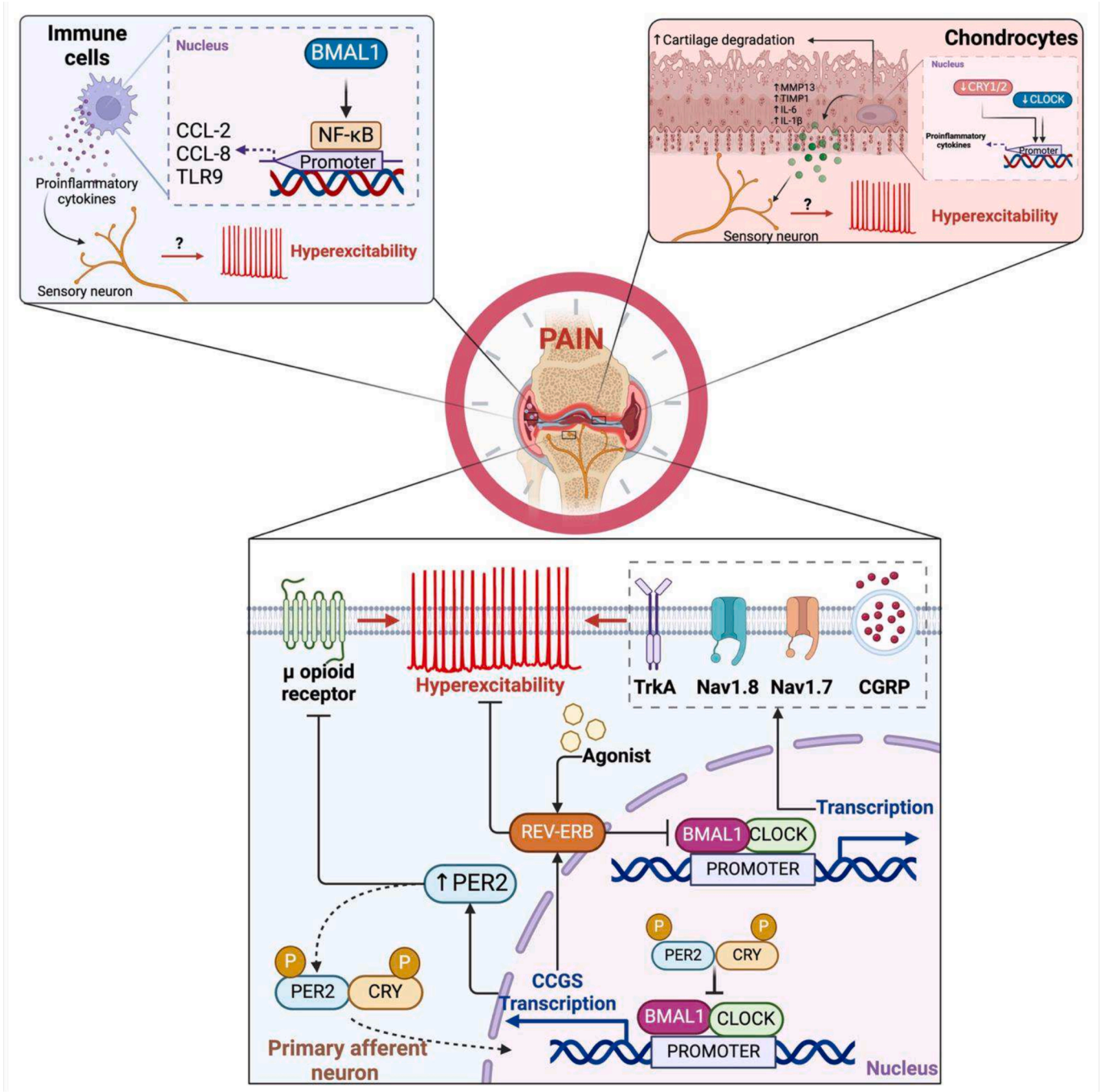

Fig. 2. Role of clock genes in OA-induced pain. BMAL1 activation increases CGRP synthesis and promotes the expression of TrkA, TRPV1, Nav1.8, and Nav1.7 in nociceptors in the DRG, promoting the development and maintenance of chronic pain. Additionally, pharmacological activation of REV-ERB, a negative regulator of BMAL1, reduces OA-induced pain by reducing BMAL1 expression. Furthermore, BMAL1 activation promotes NF-kB activation and the transcription of proinflammatory cytokines and receptors like CCL-2, CCL-8, and TLR9 in immune cells, which may converge to further maintain pain. High levels of PER2 have been associated with increased hyperexcitability induced by a reduction on the expression of the μ opioid receptor. Moreover, PER2 knockout in astrocytes results in analgesia mediated by the upregulation of α1D-AR and the consequent increase in 2-AG synthesis. Once PER2 and CRY are phosphorylated, they translocate to the nucleus to regulate the circadian cycle by inhibiting the interaction of BMAL1 and Clock. Despite some studies having demonstrated a role of BMAL1 and PER2 in pain signaling, their specific involvement in the context of OA remains unclear. Regarding other clock genes, it has been demonstrated that the downregulation of CRY1/2 and CLOCK in chondrocytes is associated with increased cartilage degradation and the release of proinflammatory molecules by chondrocytes. These molecules can sensitize the sensory neurons innervating the joint, triggering a pain response. Abbreviations are as follows: 2-AG, 2-arachidonoylglycero; α1D-AR, alpha-1D adrenergic receptor; BMAL1; brain and muscle ARNT-like protein 1; CCGS, clock-controlled genes; CCL-2, chemokine (C-C motif) ligand 2; CCL-8, chemokine (C-C motif) ligand 8; CGRP, calcitonin gene-related peptide; Clock, circadian locomotor output cycles kaput; CRY, cryptochrome; DAG, diacylglycerol; DRG, dorsal root ganglia; IL-1β, interleukin 1 beta; IL-6, interleukin 6; MMP13, matrix metallopeptidase 13; NF-kB, nuclear factor kappa-light-chain-enhancer of activated B cells; Nav 1.7, voltage-gated sodium channel subtype 1.7; Nav1.8, voltage-gated sodium channel subtype 1.8; OA, osteoarthritis; PER2, period circadian protein homolog 2; REV-ERB, nuclear receptor subfamily 1 group D member 1; TIMP1, metallopeptidase inhibitor 1; TLR9, toll-like receptor 9; TrkA, tropomyosin receptor kinase A; TRPV1, transient receptor potential vanilloid-1.

conditions. However, we also recognize that BMAL1 may affect pain sensitivity through its role in protein translation, independently of its circadian function. This is especially relevant in chronic pain contexts, where pain-related proteins are modulated over longer periods, beyond the typical 24-hour cycle. The dual role of BMAL1 in both circadian and translational regulation adds complexity, as both mechanisms may work together to fine-tune pain pathways. Thus, understanding the potential interaction between the effect of BMAL1 on circadian rhythms and protein translation interact could provide new insights into how clock genes influence pain modulation, both in terms of timing and function.

5.2. PER as a potential player in OA-induced pain

Since PER2 is expressed in the DRG, trigeminal ganglia, and the spinal cord, this gene could also play a role in the pathophysiology of OA pain (Morioka et al., 2016; Shirakawa et al., 2023; Zhang et al., 2019). Studies in naïve mice show that PER2 knockout increases the threshold to mechanical and thermal stimuli (Zhang et al., 2011), which has been associated to the upregulation of μ opioid receptor in the ventral tegmental area and elevated levels of oxytocin (Custodio et al., 2022; Zhang et al., 2011). The role of PER2 has also been explored in chronic pain conditions. For instance, PER2 deletion prevented the development of neuropathic pain through the release of 2-arachidonoyl glycerol from astrocytes in the spinal cord (Yamakawa et al., 2024). Similarly, the upregulation of PER2 in nucleus accumbens of mice was linked to opioid-induced hyperalgesia (Zhang et al., 2019). A mutation in the PER2 gene was associated with dysregulation in the circadian rhythm of tumor necrosis factor alpha (TNF-α). Although, these studies may suggest that PER2 contributes to pain modulation through several pathways, we recognize that a major limitation in proposing PER2 as target for treating OA pain is the lack of direct evidence linking this gene to OA-related pain mechanisms. Further research is needed to clarify the role of PER2 interactions in OA pain, as shown in other pain types. Although current studies are lacking, evidence of its pronociceptive effects suggests that PER2 may play a significant role in OA pain.

PER3 (Period Circadian Regulator 3) is a critical component of the circadian clock, responsible for regulating biological rhythms and coordinating various physiological processes (Liberman et al., 2017). It has been implicated in multiple genome-wide association studies related to mood and sleep disorders (Hida et al., 2014; Hu et al., 2016). Mice lacking Per3 maintain a functioning circadian clock, but their circadian period is shorter than wild-type mice, suggesting a key role in regulating timing and rhythm (Lee et al., 2004). The PER3 gene contains a variable-number-tandem-repeat (VNTR) region, consisting of four or five repeating sequences of fifty-four base pairs that encode eighteen amino acids, which is important for its function (Hida et al., 2018). Variations in the PER3 gene have been shown to influence human behavior and physiology, including diurnal preferences, sleep-wake cycles, and cognitive abilities (Viola et al., 2007). Moreover, single nucleotide polymorphisms (SNPs) in PER3 have been associated with altered circadian parameters, chronotypes, and mood disorders, including depression (Carvalho et al., 2019; Karthikeyan et al., 2014; Liberman et al., 2017). As mentioned earlier, sleep disturbances—such as deprivation or fragmentation—are known to exacerbate chronic pain. In this context, a study showed that PER3 gene polymorphisms, particularly the 4/4 genotype, are linked to fibromyalgia, suggesting that PER3 gene variations may play a role as a genetic risk factor in fibromyalgia development (Parvez et al., 2024). Furthermore, our preliminary findings suggest that hypomethylation of the PER3 gene is associated with OA pain, underscoring the need for a deeper understanding of how PER3 and other circadian genes modulate pain sensitivity. Further research is required to explore the mechanisms by which circadian genes like PER3 contribute to chronic pain conditions, which could potentially inform new therapeutic approaches targeting circadian regulation in pain management.

5.3. Potential role of other clock genes in OA pain

Cryptochrome 1/2 (CRY1/2) are clock genes involved in inflammatory pathways associated with both rheumatoid arthritis and OA. It was reported that impaired expression of CRY1/2 increases pro-inflammatory cytokines in rheumatoid arthritis (Narasimamurthy et al., 2012). Consistently, the attenuation of CRY1 expression by melatonin in a collagen-induced arthritis mouse model led to an elevation of type II collagen antibodies, infiltration of inflammatory cells, synovial hyperplasia and destruction of articular cartilage (Bang et al., 2012). Yet, there is evidence showing that CRY2 has a protective effect on cartilage in OA animal models (Bekki et al., 2020). Regarding this, CRY2 knockout mice with surgical-induced OA displayed more cartilage, subchondral bone, and synovium damage compared to their wild-type counterparts (Bekki et al., 2020). Additionally, RNA-seq analysis of healthy and OA human articular cartilage revealed that CRY2 is significantly reduced in OA, which contributes to the dysregulation of extracellular matrix-related pathways, such as PI3K-Akt, HIF-1, and FoxO, as well as circadian rhythm pathways (Fisch et al., 2018).

CLOCK is a key regulator of rhythmic activity in chondrocytes. The CLOCK and BMAL1 dimer maintains a fixed oscillation of genes involved in cartilage matrix synthesis and cartilage degradation, such as CD44, MMP13, TIMP1 and IGF-1 (Rao et al., 2014). This balance is essential for regulating anabolic and catabolic metabolism in chondrocytes. Moreover, a mutation of CLOCK resulted in high levels of inflammatory factors such as IL-6, IL-1β and MCP1, due to the overactivation of NF-κB in chondrocytes (Yuan et al., 2019).

Although these studies did not specifically explore the role of CRY1/2 or CLOCK in nociceptive behaviors, inflammation is a key driver of pain. Thus, the clear involvement of these genes in modulating inflammatory pathways strongly suggests that they could be involved in rheumatoid arthritis- or OA- induced pain. In support of this hypothesis, clock genes have been explored in other types of pain. For example, the expression of CRY1 is significantly disrupted in the lumbar spinal dorsal horn of mice following a partial sciatic nerve ligation (Morioka et al., 2016). Additionally, CLOCK regulates the rhythmic expression of *Tac1*, the gene that encodes for substance P in DRGs of mice (Zhang et al., 2012). This is achieved through the binding of CLOCK:BMAL1 heterodimers to the E-box element of the *Tac1* promoter, generating a cyclic expression of the gene throughout the day. The circadian-regulated signaling has been shown to regulate inflammatory nociceptive behaviors in mice injected with formalin (Zhang et al., 2012). Collectively, these findings suggest that, beyond their well-established roles in the cartilage metabolism and inflammation process, CRY1/2 and CLOCK may also contribute to the circadian rhythmicity of chronic pain. However, further research is needed to fully understand the involvement of these genes in OA pain and their potential as therapeutic targets.

6. Chronotherapy and pain management

The circadian rhythm of a drug target is important for determining the efficacy of a drug at different times of the day, as the expression of the target fluctuates through the day. Beyond affecting efficacy, the timing of drug administration can also impact circadian rhythms related to drug absorption, metabolism and elimination (Labrecque & Bélanger, 1991). This is especially important for drugs with short half-lives, where administrating the drug at the optimal time for target expression can maximize effectiveness.

Chronotherapy, an approach based on timing of medication administration with the circadian rhythms of body, has emerged as a promising strategy for managing OA-related pain (Bumgarner et al., 2023). In this context, a preclinical study reported that indomethacin is more effective in increasing the grip strength in rats with rheumatoid arthritis between 9:00 – 15:00 h compared to the evening (18:00 – 21:00 h) (Labrecque et al., 1995). Similarly, long-term treatment with acetaminophen and celecoxib, but not aspirin, restored nocturnal behavior

in rats with arthritic pain (Millecamps et al., 2005), suggesting circadian variations in the analgesic effects induced by non-steroidal anti-inflammatory drugs (NSAIDs).

Clinical trials have also demonstrated the effectiveness of chronotherapy in OA pain management. One trial involving 517 patients found that evening administration of indomethacin was most effective in patients with nocturnal pain, while morning or noon ingestions was optimal for those experiencing pain in the afternoon or evening (Labrecque et al., 1995; Levi et al., 1985; Martin et al., 1914). Additionally, oral administration of flurbiprofen twice daily during the night effectively reduced morning stiffness and pain in patients with rheumatoid arthritis (Kowanko et al., 1981). Collectively, these findings emphasize the importance of considering medication timing for effective pain management, highlighting the potential of chronotherapy to improve outcomes.

Alternatively, some studies have reported no fluctuations in NSAID effectiveness in patients with OA. Notably, ketoprofen or celecoxib (200 mg) demonstrated significant pain reduction without temporal variations in analgesia (Stengaard-Pedersen et al., 2004; Vinje et al., 1993). However, these studies did not consider when the arthritic pain is highest and lowest before comparing the effectiveness of NSAIDs at different times of the day, instead, patients were randomly assigned to groups, potentially masking effects that might have been observed with a more personalized approach.

Chronotherapeutic strategies have also been explored for diseases relevant to OA. For example, research into the circadian basis of morning joint stiffness and pain in rheumatoid arthritis has led to the development of modified-release glucocorticoids designed to act during the night (Cutolo, 2019). These modified-release formulations have proven more effective than immediate-release glucocorticoids taken in the morning for reducing morning stiffness in patients (Buttgereit et al., 2008). In osteoporosis, morning administration of teriparatide was found to be more effective than evening administration in improving bone mineral density over a 12-month period (Michalska et al., 2012). Additionally, due to its effects on the chondrocyte molecular clock, teriparatide has shown promise in preventing cartilage degeneration in a mouse model of OA (Sampson et al., 2011). In future clinical trial design, it will be important to consider the circadian physiology of joint tissues and evaluate the importance of treatment timing. Overall, chronotherapy presents an exciting opportunity to revolutionize pain management by harnessing the body's natural rhythms to maximize treatment effectiveness. This innovative approach promises not only better pain control but also a significant improvement in patients' quality of life.

7. Future considerations and conclusions

Pain exhibits circadian rhythms in various structures, including the DRG, spinal cord, and brain areas, but the origins of these rhythms remain unclear. It is not yet certain whether these circadian variations in pain thresholds are driven solely by the pain system itself or if they are influenced by interactions with other systems, such as the endocrine and immune systems. This complexity suggests that disruptions in specific systems could contribute to the observed differences in circadian pain thresholds across various conditions. Consequently, understanding the mechanisms underlying these rhythms in the pain system, particularly in OA, is a challenging task that requires collaborative and multidisciplinary approaches.

Further challenges in the field include the fact that many preclinical studies on pain behavior are conducted during the light phase, when nocturnal species are typically inactive, which may limit the relevance of these findings to human pain conditions. Moreover, there is a notable disparity in the sex of the animals used in these studies, with most research focusing exclusively on males. Given the established sex differences in pain perception and response, this lack of diversity may reduce the generalizability of the findings. To address these limitations, future research must include both sexes and consider the impact of circadian rhythms in both human and animal studies.

Integrating chronobiology into pain research is crucial since circadian variations in pain sensitivity underscore the importance of understanding the temporal dynamics of pain perception and modulation. This is particularly relevant for the treatment of OA pain, which is challenging *per se* due to its complex and multifaceted nature. Emerging evidence suggests that clock genes like BMAL1 and PER2 play a significant role in pain modulation by regulating proinflammatory molecules and ion channels implicated in pain signaling. Specifically, the role of BMAL1 in OA pain is relatively well-studied, while the contribution of PER2 remains less explored, presenting an opportunity for further investigation and potential therapeutic interventions.

To advance our understanding and management of OA pain, it is essential to elucidate the specific molecular pathways and cell types through which BMAL1 and PER2 (as well as other clock genes) exert their effects. Addressing these gaps will be crucial for developing targeted chronotherapy approaches that harness circadian rhythms to optimize pain management. In conclusion, integrating chronobiology and clock gene research into pain management strategies holds significant promise for improving outcomes and quality of life for patients with chronic pain conditions like OA.

Author contributions

RK, KA, and YCA conceived the idea for the article. EJR-P performed the literature search and wrote the first draft with help from SLL. EJR-P created the article figures in BioRender.com. RK, KA, and YCA edited the article. All authors critically revised the work.

Declaration of Data Availability

This declaration acknowledges that this paper adheres to the principles for transparent reporting and scientific rigor of preclinical research and that all data supporting the results are presented in the manuscript.

CRediT authorship contribution statement

Erick J. Rodríguez-Palma: Writing – review & editing, Writing – original draft, Visualization, Data curation, Conceptualization. **Santiago Loya-Lopez:** Writing – review & editing, Writing – original draft, Formal analysis. **Kyle Allen:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization. **Yenisel Cruz-Almeida:** Writing – review & editing, Writing – original draft, Conceptualization. **Rajesh Khanna:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Formal analysis, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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