

## Overview

# Interacting Influences of Sleep, Pain, and Analgesic Medications on Sleep Studies in Rodents

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This overview provides a brief summary of the complex interactions that link sleep, pain, and analgesic medications. Sleep scientists and clinicians are well aware of these relationships and understand that maintaining healthy pain-free subjects in a stable environment is essential to generating interpretable data and valid conclusions. However, these concepts and the data that support bidirectional interactions between sleep and pain may be less known to those who are not sleep scientists yet need such information to protect and advance both animal wellbeing and research validity (for example, veterinarians, IACUC members). Abundant human evidence supports the disruptive effect of pain and the modulatory effects of analgesic drugs on sleep; however, analgesic drugs can alter both sleep and the electroencephalogram, which is the primary objective measure for identifying sleep and evaluating sleep properties in both humans and animals. Consideration of the modulatory and interactive relationships of sleep, pain, and analgesic medications is essential to designing and conducting valid and reproducible sleep research using animal subjects.

**Abbreviations:** CCI, chronic constrictive injury; EP, evoked potential; NREMS, non-rapid-eye-movement sleep; PS, paradoxical sleep; REMS, rapid-eye-movement sleep; SWS, slow-wave sleep

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## General Considerations for Studying Sleep in Research Animals

The purpose of this overview is to provide a succinct summary of the complex interactions linking sleep, pain, and analgesic medications to those who are not sleep scientists yet may need such information to protect and advance both animal wellbeing and research validity (for example, veterinarians, IACUC members; Figure 1). The general mechanisms underlying sleep neurophysiology and the algorithms for identifying and characterizing sleep have been well summarized previously and will not be discussed here; interested readers are referred to many available overviews for this information (for example, references 7, 12, 54, 59, and 74). However, terminology may require a brief explanation. Sleep is generally classified into 2 basic forms: nonrapid-eye-movement sleep (NREMS), with the deepest stages also known as slow-wave sleep (SWS), and rapid-eye-movement sleep (REMS; also known as paradoxical sleep [PS]). Both designations are found in the literature, and for purposes of the current discussion, readers can generally view these alternate names as synonymous. However, to prevent confusion related to literature referenced later, readers should be aware that although these designations can refer to both preclinical (animal) and clinical (human) sleep, clinical assessment of sleep in human medicine and research generally involves subclassification of NREMS (SWS) into the classic categories of sleep

stages 1 through 4 or, as recently redefined, as sleep stages N1 through N3<sup>13,40</sup> (Figure 1).

The technical method used to monitor sleep can influence the potential pain experienced by the animal subjects and the associated need for analgesic medication or other interventions. Four approaches have commonly been used to monitor sleep in rodents: observation (video analysis), piezoelectric films, telemetry, and tethering. Observation and piezoelectric films do not require surgical preparation of the animal. In the piezoelectric method, mice are housed directly on the films, which can detect animal movements with high sensitivity.<sup>42,81</sup> The regularity of the output signal related to breathing movements associated with sleep, in the absence of signals related to overt animal movement, can be used to assign conditions of sleep or waking.<sup>42,81</sup>

These 4 approaches to monitoring sleep each have various advantages and disadvantages that will not be reviewed here, except as relevant to the topic of pain. The noninvasive approaches of observation and piezoelectric films are less likely to be associated with pain than are telemetry and tethering, which require surgery. However, the parameters that underlie the piezoelectric method—body movement and respiration—are confounded in studies that involve pain or analgesia because pain, analgesia, and the associated experimental models can substantially change body movements and respiration without necessarily altering sleep. Furthermore, although observational and piezoelectric approaches can capture some aspects of sleep (for example, total sleep time), other features are not discernable without implanted cranial electrodes (for example, EEG

1. Sleep researchers are intensely aware that maintaining comfortable pain-free subjects in a stable environment, as consistent with the experimental goals, is essential to generating interpretable data and valid conclusions.
2. The technical method used to monitor sleep can influence the potential for postoperative pain and the associated need for analgesic medication or other interventions.
3. Bidirectional relationships linking pain and sleep are well known to scientists who study sleep in animals and in people.
4. Pain can disrupt subsequent or on-going sleep, and sleep disruption can exacerbate or precipitate pain.
5. Many analgesic drugs are known to modify the production of endogenous sleep mediators, the amount or characteristics of sleep itself, or the EEG, which is the fundamental element on which sleep scoring relies.
6. The use of analgesic agents in preclinical research can confound interpretation of how pain or an associated disease condition influences sleep.

**Figure 1.** Key points.

power in various frequency ranges). Therefore, surgery may be essential for studies that require detailed EEG-based assessment of sleep stages and other properties. The approach used by individual investigators largely reflects the experience of the laboratory, the available data collection equipment, and the nature of the research question being asked.

Telemetry and tethering approaches to sleep monitoring both require surgery. However, tethering requires only cranial surgery, whereas telemetric monitoring typically involves concurrent abdominal surgery (cranial implantation of EEG electrodes that are tunneled subcutaneously to an abdominally implanted telemetry transmitter). The clear advantages of telemetry over tethering are that a cable does not restrict animals, the animals can be monitored in standard caging, and transfer of animals to other test devices (for example, a rotarod or open field) is simplified;<sup>58</sup> in addition, detachment of the cable to allow animal manipulation or relocation can sometimes loosen headpieces or alter characteristics of the EEG after reattachment. A comparison of sleep data from mice monitored by telemetry with previously published data collected using a tether system indicated that the light- and dark-phase sleep durations obtained with telemetry differed somewhat from those obtained with tethers, due mainly to more sleep during the dark (active) phase in the telemetry recordings.<sup>69</sup> Photobeam interruption in the home cage has been used to compare preoperative and postoperative locomotor activity in mice that were surgically instrumented for either tethered or telemetric sleep monitoring.<sup>68,69</sup> The data showed significant reductions in activity after surgery in both situations, with mice instrumented for telemetry showing greater reductions in activity than those instrumented for tether recording.<sup>68,69</sup> However, the data collection times relative to surgery were not specified in these studies, and data were not presented to address the chronicity of the reduction in activity (that is, a temporary change associated with surgical recovery time or a long-lasting reduction due to the presence of the abdominal transmitter or tether).<sup>68,69</sup> A study that evaluated the influence of various tether configurations on activity in mice found that reductions in activity occurred in a graded manner as cable weight increased and cable flexibility decreased; the time periods at which measurements were taken before and after surgery were not specified.<sup>68</sup> Taken together, these findings suggest that telemetry surgery, which requires both cranial and peripheral incisions, may require longer recovery times than surgery for tethering, which requires a single surgical site. However, this possible disadvantage is likely balanced by the ability to avoid restrictive cabling and to use standard caging in telemetry studies. Furthermore, the telemetry approach allows comparison of sleep in individual animals maintained in either group or individual housing situations.<sup>21</sup>

Research programs that use animals (or people) to investigate sleep can study normal sleep and its mechanisms, sleep in the context of a human health condition, and the modulatory impacts of sleep disruption or loss on health and disease. In

studies of sleep in disease models that are secondarily associated with pain, the pain may either be an inherent part of the model or a confound that requires management. For example, in studies of sleep during inflammatory conditions, associated pain may confound clear interpretation of the direct effects of inflammatory mediators on sleep, or pain may be viewed as part of the inflammatory syndrome.<sup>29,30</sup> Furthermore, sleep disruption can exacerbate pain (reviewed later). Although human patients may receive pain-relieving medications in association with painful medical conditions, the use of such agents in preclinical research can confound the interpretation of how pain or the associated disease condition influences sleep. This confound arises because some analgesic medications are antiinflammatory and thus may interfere with the generation of mediators that are likely to alter (promote or inhibit) sleep,<sup>30</sup> whereas other types of analgesics may interact with neural sleep circuitry in the brain and thereby potentially influence sleep in that manner (reviewed later). Therefore, the selection of a suitable analgesic regimen is complex. Most researchers now use perioperative analgesia, with the expectation that these agents will be discontinued days or weeks before experimental data collection begins. However, the use of analgesic medications during data collection in some experimental studies may be contraindicated based on the purpose of the study. For example, in a study of experimentally induced arthritis and sleep, the use of antiinflammatory drugs would influence both the generation of sleep mediators and disease expression, thereby potentially confounding the data or its interpretation. In such cases, the IACUC must carefully weigh the magnitude of the pain involved, the potentially confounding effects of either pain or analgesics, and the merits of the study. Furthermore, investigations of the complex interactions that link sleep, pain, and analgesia should be conducted with careful attention to details of model, experimental design, and outcome measures that will maximize their translational predictability, validity, and scientific value.<sup>47</sup>

### **Pain and Sleep: Bidirectional Relationships**

Extensive data from human studies, collected from patients and volunteers in respective clinical and experimental settings, from self-report and objective polysomnographic study designs and in longitudinal and cross-sectional investigations support bidirectional and mechanistic relationships between sleep and pain (reviewed in references 11, 14, 16, 17, 22, 23, 24, 34, and 53). These bidirectional relationships are well known to scientists who study sleep in animal models, and sleep researchers recognize that using healthy pain-free well-controlled subjects as models of human disease is essential to generating interpretable data and valid conclusions.<sup>9</sup> The current overview provides only a limited survey of this extensive field of research by using a few select examples.

**Pain disrupts sleep.** Human studies are highly relevant to considerations of how unmitigated pain is likely to modify the normal sleep patterns of animals used in studies of sleep. The

prevalence of sleep disturbance in patients with chronic pain reportedly ranges from 50% to 80%, and in general, the severity of sleep disturbance is related to pain intensity.<sup>14,17,34,60,64,65,78</sup> In patients with either acute pain or burn pain, back pain, and other forms of chronic pain, a day with high pain can be followed by a night of poor sleep.<sup>1,11,14,34,48,62</sup> For example, a study of 50 women with fibromyalgia who recorded their sleep quality, pain intensity, and attention to pain for 30 d found that a more painful day was followed by a night of poorer sleep, a night of poor sleep was followed by a more painful day, and poorer sleepers tended to report significantly more pain.<sup>1</sup> In contrast, another study interviewed 28 nonventilated burn patients on 5 consecutive mornings, with 75% reporting disturbed sleep.<sup>48</sup> Regression analyses of these data indicated that a night of poor sleep was followed by a significantly more painful day, although pain during the day was not a significant predictor of poor sleep on the following night in this study.<sup>48</sup> As these 2 examples illustrate, the relationships between pain and sleep can vary depending on many factors, including the environment and the type and severity of pain. Furthermore, human subjective perceptions of sleep (for example, depth of sleep, duration of sleep, number of awakenings, daytime sleepiness) can be discordant with objective assessments.<sup>10,28,43,55,77</sup> Although any type of pain has the potential to disrupt sleep, the types and gradations of pain that have been studied in this capacity are often clinically associated with specific diseases, health conditions or types of pain in humans. Finally, individual variation is substantial in human populations. This variation has led to suggestions that in the assessment of pain and analgesia, a focus on within-subject changes rather than group-level averages merits greater importance in clinical studies and perhaps in preclinical studies as well.<sup>46,47</sup>

In human polygraphic studies, sleep architecture, generally defined as the percentage of time spent in each sleep stage, is not consistently different between patients with chronic pain and control subjects across studies.<sup>10,11</sup> However, in patients with chronic pain, sleep continuity is often fragmented, with frequent microarousals (3 to 10 s long, involving transient brain, heart and muscle activations), awakenings (activations lasting longer than 10 to 15 s, with possible consciousness), shifts in sleep stage (for example, from a deeper to a lighter sleep stage), body movements, or some combination of these effects.<sup>11,53</sup> An experimental polygraphic study of 13 healthy adults found that noxious hypertonic infusions triggered significantly more awakenings and led to lower sleep quality than did the nonnoxious control infusions and that the painful stimulus was found to be disruptive in all stages of sleep.<sup>35</sup>

Studies of the effects of pain on sleep in animals have similar findings to those from human studies. The animal studies typically involve baseline sleep recording, followed by induction of an experimental painful condition and evaluation of subsequent effects on sleep, with each animal providing its own baseline data. Several examples illustrate this design. In one study, mice were instrumented with telemeters to record the EEG and body temperature; sleep monitoring began before and continued after the induction of musculoskeletal sensitization by 2 injections of acidified saline into the gastrocnemius muscle.<sup>66</sup> Although the experimental sensitization did not cause significant alterations in the amount of time spent in wakefulness or sleep, the experimental mice showed fragmentation of sleep and alterations in the EEG spectra during the light (somnolent) phase of the diurnal cycle.<sup>66</sup> Another study examined the long-term effects of chronic articular pain on sleep in rats with experimentally induced osteoarthritis.<sup>61</sup> Alterations in sleep were observed during

both light and dark phases of the diurnal cycle, beginning on day 1 and continuing until the end of the study.<sup>61</sup> Arthritic rats showed fragmented sleep, reduced sleep efficiency, less NREMS and REMS, and fewer bouts of REMS, with males more affected than females.<sup>61</sup> A third study investigated the changes of sleep parameters in rats with chronic constriction injury (CCI), modeling neuropathic pain.<sup>72</sup> Six days after CCI surgery, the rats exhibited both mechanical allodynia and neuropathic pain.<sup>72</sup> The EEG in the frontal cortex of CCI rats and the EMG were measured over 6 h, during which rats were placed on sandpaper as an aversive condition for comparison with placement on bedding.<sup>72</sup> Rats placed on bedding showed no significant difference between CCI and sham-operated groups in sleep latency, total waking time, total NREMS time and total REMS time.<sup>72</sup> In contrast, CCI rats placed on sandpaper showed a significant delay in sleep onset and an increase in total waking time as compared with the sham group, although the 2 conditions were not associated with significant differences in total NREMS or total REMS times.<sup>72</sup> Another study used the EEG to objectively assess sleep in association with 4 types of pain (inflammatory, neuropathic, postoperative, and osteoarthritic) in rats.<sup>37</sup> The amplitude of 1- to 4-Hz waves and time spent in stage 2 sleep were significantly lower in all models except osteoarthritis, indicating that sleep disruption was more likely to affect the deeper stages of sleep.<sup>37</sup> In addition, pain can influence the EEG in a manner that could complicate scoring of sleep. For example, as compared with the naïve state, rats with experimental acute, inflammatory, or neuropathic pain developed greater EEG power and corticocortical coherence in the primary somatosensory and prefrontal cortex.<sup>36</sup> An important consideration in these types of animal studies, as compared with most human studies, is that the effects of pain on sleep in preclinical models typically are measured during a relatively acute phase of the painful condition (hours or days); therefore, the mechanisms underlying the both pain and the modulatory effects of analgesic medications may differ from those in human patients, who generally have experienced the painful condition over a long period of time (weeks, months or years).<sup>32,47</sup>

The potential pain associated with the postsurgical period in rodents mandates careful attention to using good surgical technique and allowing adequate recovery before starting data collection for sleep studies. In human studies, surgical patients frequently experience postoperative sleep problems that include sleep fragmentation, reduced total sleep time, and reduced time spent in NREMS and REMS.<sup>15</sup> The cause and effect in these situations are difficult to unravel because hospital environments, with their inherent noise, light, and traffic, are notoriously disruptive for sleep, disturbed sleep can exacerbate pain, and both pain and analgesic treatments have the capacity to disrupt sleep.<sup>15</sup> However, surprisingly little information is available in the literature documenting the minimal recovery times for mice or rats after surgery for sleep monitoring. One such study measured sleep and EEG spectra during the 14- to 15-d period after surgical instrumentation of rats for telemetric monitoring, with ibuprofen provided in the drinking water for 3 d before through 3 d after the procedure.<sup>71</sup> Sleep, activity, and EEG spectral power were measured via telemetry on days 1, 2, 3, 7, 14, and 15 after implantation surgery.<sup>71</sup> As compared with values obtained on days 14 and 15, significant differences in NREMS and REMS times, total sleep time, EEG power, and locomotor activity were noted on days 1 through 3 after surgery; stabilization of REMS time required over 7 d.<sup>71</sup> However, the influences of pain and analgesia cannot be differentiated with this experimental design.

Several studies have used previously implanted telemetry devices to assess potential pain after subsequent secondary abdominal surgery with and without administration of analgesic medications in mice.<sup>6,27</sup> In response to the secondary surgery, these studies generally find either no postoperative changes in monitored parameters or changes that persist for hours to a few days and that are small to moderate in magnitude, with some modulatory effects of perioperative analgesic medications.<sup>6,27</sup> However, the effects on the mice in these secondary surgeries are likely less severe than those associated with abdominal implantation of telemetry devices, either alone or in combination with cranial surgery to allow recording of the EEG. Reported postoperative recovery and stabilization periods in telemetry studies range from 2 to 6 wk in various studies.<sup>58</sup> In tethered preparations, animals generally are not connected to the tether until the electrode implantation site has healed, to reduce the likelihood of both tether-related pain and dislocation of the cranial implant during early stages of healing. In contrast, telemetry easily allows documentation of sleep stabilization after surgery. Presumably, individual laboratories validate adequate recovery from surgery and associated medications before beginning experimental use of research animals. Publication of such data in the peer-reviewed literature would be valuable to the field.

**Disrupted sleep can precipitate or exacerbate pain.** Complementing the human studies showing that pain disrupts sleep are numerous studies showing that poor or disrupted sleep precipitates or exacerbates pain.<sup>1,2,34,48,62</sup> From a different perspective, a 4-y prospective longitudinal study of 2761 patients from general medical practices found that good quality sleep was a statistically independent predictor of musculoskeletal health in this population.<sup>31</sup>

Sleep deprivation and disruption are particularly prevalent in patients in a critical care environment.<sup>5,25</sup> Numerous observational studies have demonstrated that the sleep of patients in ICU is highly abnormal, yet little is known about the effects of poor sleep quality on outcomes from critical illness or injury.<sup>25</sup> Reasons for sleep deprivation in the ICU are multifactorial; major contributing factors include the type and severity of underlying illness, the pathophysiology of acute illness or injury, pain from surgical procedures, and perhaps most importantly, the ICU environment itself.<sup>5,25</sup> Sleep in ICU patients is characterized by prolonged sleep latencies, sleep fragmentation, decreased sleep efficiency, frequent arousals, a predominance of stage 1 and 2 NREMS, decreased or absent stage 3 and 4 NREMS, and decreased or absent REMS.<sup>25</sup>

Numerous experimental studies with healthy human subjects have attempted to delineate these relationships, as illustrated by the following examples. One study subjected 17 healthy adults to 3 wk of restricted sleep with limited recovery (5 nights of 4-h sleep per night followed by 2 nights of 8-h sleep per night) as compared with a control protocol in which participants were permitted 3 wk of 8-h sleep per night.<sup>62</sup> Compared with the control period, the sleep-restriction period was associated with mild increases in spontaneous pain, transient reductions in thermal pain thresholds, and decreased habituation to cold pain.<sup>62</sup> The authors concluded that chronic exposure to insufficient sleep could increase pain vulnerability by altering processes of pain habituation and sensitization.<sup>62</sup> A 7-night polysomnographic study evaluated the effects of partial sleep loss on reports of spontaneous pain in 32 healthy women.<sup>64</sup> On nights 1 and 2 (baseline), subjects slept undisturbed for 8 h.<sup>64</sup> After night 2, subjects were randomized to control conditions (continued undisturbed sleep), sleep disruption (1 forced awakening per

hour over 8 h of sleep), or sleep deprivation (same total sleep time as subjects with forced awakenings, achieved by delayed bedtime).<sup>64</sup> Disrupted and deprived subjects both demonstrated 50% reductions in total sleep time, increases in nonpainful somatic symptoms, and an increase in spontaneous pain.<sup>64</sup> These authors concluded that loss of sleep continuity, rather than simple loss of sleep, impairs endogenous pain-inhibitory processes and promotes spontaneous pain, thus supporting a possible pathophysiologic role of sleep disturbance in chronic pain.<sup>64</sup>

Several studies have addressed these relationships using animal models. Investigators generally use 1 of 3 approaches to investigate how sleep disruption affects pain in rodents: fragmentation of sleep, total sleep deprivation, and REMS deprivation. With regard to fragmentation, one study in mice found that 5 d of light-phase sleep fragmentation during the development of musculoskeletal sensitization exacerbated subsequent pain responses, with persistent effects on NREMS and sleep-wake transitions.<sup>67</sup> Another study reported that moderate daily sleep loss, but not sleep fragmentation, increased sensitivity to noxious stimuli and exaggerated pain responses in healthy mice without general sensory hyperresponsiveness.<sup>4</sup> Administration of the arousal-promoting agents caffeine or modafinil, which had no analgesic activity in rested mice, normalized pain sensitivity in sleep-deprived mice.<sup>4</sup> The reversibility of mild sleep-loss-induced pain by wake-promoting agents led the authors to propose that increased alertness, as well as adequate sleep, could reduce pain sensitivity.<sup>4</sup> The different pain modalities measured in these 2 studies<sup>4,67</sup> and the different methods and extent of sleep disruption that were used preclude direct comparisons of specific conclusions. However, both studies reveal substantial effects of sleep disruption on pain.<sup>4,67</sup> Another group produced sleep disruption in rats by placing them for 8 h in a slowly rotating cylindrical cage that repeatedly caused arousal by invoking the righting reflex.<sup>80</sup> This treatment caused robust mechanical and thermal hypersensitivity without changing plasma corticosterone levels.<sup>80</sup>

Several studies have focused on effects of REMS deprivation on pain, probably in part because this status has historically been technically easier to achieve over a longer time than has total sleep deprivation.<sup>34</sup> However, some approaches to causing REMS loss in rodents, particularly the 'pedestal over water' and 'inverted flower pot' methods, may have nonspecific effects that could significantly affect pain outcome measures, despite attempts to appropriately control for the environment.<sup>44,45</sup> Furthermore, isolated loss of REMS is a not common condition in people or animals, although alterations in REMS have been associated with some human health conditions.<sup>39,44</sup> Nonetheless, studies have generally found that REMS deprivation increases nociceptive behavior and impairs the analgesic action of endogenous and exogenous opioids.<sup>34,73,76</sup> Pre- or postsurgery exposure of rats to REMS disruption for 6 h daily on 3 consecutive days did not alter basal responses to mechanical, heat, or cold stimuli but did delay recovery from incision-induced reductions in paw withdrawal threshold to mechanical stimulation.<sup>76</sup> The authors concluded that this short-term REMS disturbance did not alter basal pain perception but did exacerbate postsurgical pain hypersensitivity.<sup>76</sup>

With regard to rodents in a vivarium setting, periodic short-term disturbances like cage changing generally have relatively transient effects on sleep.<sup>21,52,70</sup> Minor and occasional disruptions of sleep are likely to have little effect on animal or human wellbeing, given that sleep is very resilient to small or transient disruptions,<sup>33</sup> and animals in a normal research environment generally have few impediments to getting as much sleep as

they need or want. Nonetheless, even seemingly minor disruptions of sleep associated with animal husbandry do have the potential to alter the distribution and continuity of sleep, if not its total amount. The possible effect of these disruptions on disease models is an important consideration for animals on study.

In people, work requirements, family or social considerations, and even illness (for example, sleep apnea) can result in chronic or repeated states of insufficient or disrupted sleep. Similarly, experimental studies that use automated sleep deprivation or disruption methods can create a greater duration and severity of sleep disturbance than would typically occur under natural circumstances for people or research rodents (for examples, see references 20, 49, 50, 63, and 75). Such severe or chronic sleep disruption could potentially exacerbate otherwise minor pain that animals would experience if used in a pain-associated protocol (for example, a study of the effects of sleep fragmentation on experimental arthritis).

### Analgesic Medications and Sleep

Research concerns about using analgesic drugs during the collection of sleep data arise because these drugs can potentially modify both sleep itself and the EEG, regardless of the presence or absence of pain or the analgesic efficacy of the drug.<sup>14,18,22,41,56</sup> These types of pharmacologic effects could alter sleep scoring and data analysis and thus lead to erroneous or confounded conclusions relevant to the hypothesis of the study. Physicians and scientists who study human sleep generally try to identify treatment options that will both reduce pain and improve sleep quality, regardless of mechanism,<sup>22</sup> whereas preclinical sleep studies often require treatments that will reduce or alleviate pain without modifying the underlying mechanisms that influence sleep. This balance can be difficult to achieve, and for this reason, human studies of analgesics and sleep may have limited use for extrapolation to animal studies despite potential analgesic efficacy.

A review of the field of pharmacoencephalography, which studies how drugs change the normal EEG, evaluated 15 articles on the effects of analgesics on spontaneous EEG and 55 papers on effects on evoked potentials (EP) in humans.<sup>41</sup> Overall, opioids increased activity in the  $\delta$  band of the spontaneous EEG, with some increases also seen in higher frequency bands; EP amplitudes were decreased in the majority of studies.<sup>41</sup> In an actigraphic and polysomnographic study of patients with chronic back pain, several patients taking high doses of opioids had abnormal hypnograms, with deficits in deep sleep and REMS.<sup>51</sup> Conversely, disturbed sleep was one of the risk factors associated with a worse response to opioid analgesia in pediatric oncologic patients.<sup>38</sup> Reviews of literature on the effect of prescription opioids on sleep quality and efficiency find conflicting evidence regarding the benefit of opioids in improving sleep quality, duration and efficiency, with some studies suggesting a beneficial effect of opioids on sleep, and other studies demonstrating the opioids can cause sleep disturbance leading to hyperalgesia.<sup>14,22</sup> In pharmacoencephalographic studies, tricyclic antidepressants increased activity in the  $\delta$ ,  $\theta$ , and  $\beta$  bands of the spontaneous EEG, with inconsistent effects on EP.<sup>22,41</sup> Weak analgesics generally produced a decrease in EP amplitudes.<sup>41</sup> Anticonvulsants used as analgesics showed inconsistent effects on the EEG and EP but can improve neuropathic pain and have a positive effect on comorbid sleep disturbances.<sup>22,41</sup> Ketamine increased  $\theta$  band power in the spontaneous EEG and decreased EP amplitudes.<sup>41</sup> The prevalence of drug-induced changes in both human and rodent EEG has led to the suggestion that carefully controlled collection and assessment of drug-induced

changes in the rodent EEG and sleep could provide a useful biomarker for making early decisions about moving drugs from preclinical into clinical development, particularly for centrally acting drugs.<sup>7,18,19,57,79</sup>

In most preclinical studies, sleep and its stages are scored based largely based on the EEG. However, some drugs can dissociate the EEG from its normal relationship to sleep-waking behavior, confounding the interpretation of sleep studies.<sup>18,56</sup> For example, benzodiazepines increase EEG  $\beta$  power, which is a characteristic of conscious waking, during periods of sleep, whereas scopolamine and other anticholinergics can produce slow waves in the EEG even though the animals are awake.<sup>18</sup> A number of studies have shown that analgesic drugs can alter the rodent EEG in the presence or absence of pain. One study found increased EEG cortical power in somatosensory and prefrontal cortex in awake, freely behaving rats with acute, inflammatory, and neuropathic pain.<sup>36</sup> Pregabalin and mexiletine reversed the changes in power and coherence in inflammatory and neuropathic pain models, whereas EEG power was unaffected by ibuprofen in an acute pain model.<sup>36</sup> In pain-free male Sprague–Dawley rats, intravenous administration of a nonnociceptive dose of buprenorphine delayed sleep onset, significantly increased wakefulness and reduced NREMS and REMS, and increased EEG  $\delta$  power during NREMS.<sup>26</sup> related to this, a study of 8 inbred strains of rats found significant strain-associated differences in the analgesic effect of buprenorphine, with one strain designated as a hyperresponder, and 2 strains showing tail withdrawal latencies that were not significantly affected by buprenorphine administration.<sup>8</sup> Although the cited study did not evaluate sleep, it nonetheless illustrates the possibility of major strain-related response differences in analgesia—and potentially, by analogy, in EEG properties and sleep—that can influence both clinical goals and research outcomes. In rats treated bilaterally with complete Freund antigen, morphine, gabapentin, or diclofenac all showed improved sleep at doses that did not significantly alter SWS in control rats.<sup>37</sup> Effects on sleep and the EEG can also vary with the duration of drug treatment. Ketamine, for example, produces different changes in both sleep and the cortical EEG in rats, depending on whether the drug is administered acutely or chronically.<sup>3</sup> Finally, effects of analgesic drugs on sleep and the EEG may be modified by sleep disruption. For example, 8 h of sleep disruption in rats was associated with robust mechanical and thermal hypersensitivity; ibuprofen and amitriptyline attenuated mechanical and thermal effects, respectively,<sup>80</sup> suggesting dissociable mechanisms for the 2 pain modalities.<sup>80</sup>

In any study, nonanalgesic interventions should be considered and implemented whenever possible to minimize, preempt, or mitigate rodent pain and discomfort. Such interventions include providing appropriate housing, handling, and restraint; the skilled performance of procedures, including surgery; habituation to handling and other procedures; and husbandry modifications such as easier access to food and water and provision of nesting materials. Providing highly palatable or moistened food and supplemental fluids can also benefit animal wellbeing, particularly during the perioperative period after cranial surgery.<sup>58</sup>

### Summary and Perspectives

This article provides a succinct overview of the complex interrelationships that link sleep, pain, and analgesic medications (Figure 1), particularly for readers who are not sleep scientists yet may need an understanding of such information to protect and advance both animal wellbeing and research validity. As reviewed here, abundant evidence in people and animals

Correlated covariation	Problems with sleep lead to proportionally severe problems with pain Severity of pain leads to proportionally severe problems with sleep
Threshold effects	Sleep disruption must reach a threshold of severity or chronicity before triggering or exacerbating pain Pain must reach a threshold severity or chronicity before disrupting sleep
Partial, disproportionate, or damped covariation	Disruptions in sleep produce relatively smaller effects on pain Changes in pain lead to relatively smaller changes in sleep
Time lag in covariation	Variations in sleep cause time-delayed alteration in pain (for example, a bad night of sleep causes more pain the next day) Alteration in pain severity cause time-delayed alterations in sleep (for example, a painful day causes worse sleep during the subsequent night)

**Figure 2.** Potential temporal and quantitative relationships between sleep and pain. Modified with permission from reference 33.

documents the disruptive effect of pain on sleep, and disruption of sleep can promote both initiation and exacerbation of pain. The latter relationship could cause particular concern if animals that are experiencing painful conditions are experimentally exposed to sleep disruption.

Sleep researchers generally recognize that maintaining healthy pain-free subjects in a stable environment is essential to generating interpretable data and valid conclusions; however, they are also cautious about the use of pharmacologic agents that alter the EEG, sleep, or both. Careful consideration of research goals and the relevant literature is essential to arriving at an acceptable risk-to-benefit balance between pain and analgesia in preclinical studies of sleep. Furthermore, current investigations largely leave open the questions of how severe or prolonged interruptions of sleep must be to affect pain and, conversely, how severe or prolonged pain must be to alter sleep.<sup>33</sup> For example, if concordance between sleep and pain is relatively weak, then major sleep disturbances may be necessary to affect pain, such that minor day-to-day variations in nocturnal sleep quality or quantity may have no effect on pain (Figure 2).<sup>33</sup> Similarly, minor or transient pain may not adversely affect sleep but may sensitize subjects to disturbed sleep in association with other sleep impediments (for example, a noisy environment). In other words, sleep and pain may be disconnected as long as sleep remains within normal, nonpathologic limits.<sup>33</sup> In all cases, studies of the complex interactions that link sleep, pain, and analgesia should be conducted with careful attention to details of model, experimental design, and outcome measures that will increase their translational predictivity, validity, and scientific value<sup>47</sup> (Figure 2).

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