
Special Section on 50 Years of Opioid Research – Minireview

Bidirectional Relationship between Opioids and Disrupted Sleep: Putative Mechanisms

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ABSTRACT

Millions of Americans suffer from opiate use disorder, and over 100 die every day from opioid overdoses. Opioid use often progresses into a vicious cycle of abuse and withdrawal, resulting in very high rates of relapse. Although the physical and psychologic symptoms of opiate withdrawal are well-documented, sleep disturbances caused by chronic opioid exposure and withdrawal are less well-understood. These substances can significantly disrupt sleep acutely and in the long term. Yet poor sleep may influence opiate use, suggesting a bidirectional feed-forward interaction between poor sleep and opioid use. The neurobiology of how opioids affect sleep and how disrupted sleep affects opioid use is not well-understood. Here, we will summarize what is known about the effects of opioids on electroencephalographic sleep in humans and in animal models. We then discuss the neurobiology interface

between reward-related brain regions that mediate arousal and wakefulness as well as the effect of opioids in sleep-related brain regions and neurotransmitter systems. Finally, we summarize what is known of the mechanisms underlying opioid exposure and sleep. A critical review of such studies, as well as recommendations of studies that evaluate the impact of manipulating sleep during withdrawal, will further our understanding of the cyclical feedback between sleep and opioid use.

SIGNIFICANCE STATEMENT

We review recent studies on the mechanisms linking opioids and sleep. Opioids affect sleep, and sleep affects opioid use; however, the biology underlying this relationship is not understood. This review compiles recent studies in this area that fill this gap in knowledge.

Introduction

Every day in the United States, there are over 130 deaths due to opioid overdose (Hedegaard et al., 2020). The associated healthcare, lost productivity, and criminal justice involvement costs approximately \$80 billion per year (Scholl et al., 2018). Opioids are so addictive that 10%–30% of patients that are prescribed opioids misuse them and/or develop an opioid use disorder (Vowles et al., 2015). Intense withdrawal symptoms can prevent successful long-term abstinence from opioids. One such contributing opiate withdrawal symptom is disrupted sleep (Beswick et al., 2003). Poor sleep is a symptom of both opioid use (Hartwell et al., 2014) and withdrawal (Sharkey et al., 2009) and is a reason for relapse back to opioid use

(Maulik et al., 2002; Lydon-Staley et al., 2017). Specifically, more than 75% of patients on prescription opioids or methadone maintenance therapy were categorized as poor sleepers (Stein et al., 2004; Hartwell et al., 2014). Disrupted sleep leads to increases in negative affect (Sagaspe et al., 2006) and poorer cognitive abilities (Durmer and Dinges, 2005; Lim and Dinges, 2008), both of which have been associated with increased tendency to relapse. In addition, sleep disruption can lead to decreased pain tolerance (Roehrs et al., 2006), and if this occurs during opioid withdrawal, it could also contribute to high relapse rates. There may be a common vicious cycle of opioid use that induces sleep disturbances, further increasing usage of opioids. For example, poor sleep can be a risk factor for drug use, especially in adolescents. In a study of 60,000 adolescents, poorer sleep was associated with a higher odds ratio for risk of drug use (Weaver et al., 2018), and adolescents with circadian abnormalities (with secondary sleep disturbances) have increased vulnerability for addiction (Logan

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ABBREVIATIONS: A2AR, adenosine 2A receptor; CTOP, D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH₂; D1R, dopamine D1 receptor; D2R, dopamine D2 receptor; DREADD, designer receptor exclusively activated by designer drugs; EEG, electroencephalography; KOR, κ -opioid receptor; MOR, μ -opioid receptor; MPRF, medial pontine reticular formation; NAc, nucleus accumbens; NREM, non-REM; PDYN, prodynorphin; PVT, paraventricular nucleus of the thalamus; REM, rapid eye movement; RMTg, rostromedial tegmental nucleus; SWS, slow-wave sleep; TH, tyrosine hydroxylase; VLPO, ventrolateral preoptic area; VTA, ventral tegmental area.

et al., 2018). Understanding the biology linking sleep and opioids as well as targeting therapeutics to improve sleep during opioid withdrawal are necessary to help reduce the burden of opioids on society.

Currently there are few studies examining opioid use and sleep, and any mechanistic studies that do exist focus on the *acute* effects of opioids on sleep and wakefulness. However, recent technologies, such as optogenetics, chemogenetics, and fiber photometry, can be combined with electroencephalography (EEG) to give a more detailed picture of specific cell types within the brain that modulate sleep and wakefulness (summarized in Table 1). Many of these studies show that brain regions and neurotransmitter systems associated with reward greatly contribute to arousal and may mechanistically contribute to the sleep/wake state of an animal. These studies thus provide potential targets for opioid regulation of sleep states.

Effects of Opioids on Human Sleep

One of the earliest descriptions of opioid effects on sleep in humans involved four researchers who examined the effects of heroin on their own sleep. Heroin rapidly converts to morphine upon transit across the blood-brain barrier. After three nights of baseline sleep, EEG recordings were made after subcutaneous injection of heroin before sleep for three or seven consecutive nights (Lewis et al., 1970). Acute administration of heroin resulted in suppression of rapid-eye-movement (REM) sleep by 50% with smaller reductions after consecutive nights of heroin administration. Upon withdrawal, REM sleep rebound was present and persisted for days. Additionally, heroin administration suppressed deep non-REM (NREM) sleep [also known as slow-wave sleep (SWS)] while increasing both lighter NREM sleep and transitions to wakefulness and drowsiness (Lewis et al., 1970). A similar acute effect of suppressing deep NREM sleep and increasing light sleep has been seen for both long-acting morphine and methadone (Dimsdale et al., 2007). Effects appear quite similar for acute usage of short-acting morphine. Specifically, intravenous administration of morphine sulfate to healthy young adult

TABLE 1

Manipulation of opioid/reward-related neurons and their effects on sleep. A table of recent studies that manipulate neurons in reward-related brain regions and monitor sleep stages with electroencephalography. Optogenetic and chemogenetic manipulations allow exploration in a cell type-specific manner of whether certain neurons are wake-promoting or sleep-promoting.

Brain Region	Cell Type	Technique	Activate + or Inhibit -	Sleep Effect	Author	
NAc	D1R	Optogenetics	+	↑ wake	Luo et al., 2018	
		DREADDs	+	↑ wake		
		DREADDs	-	↑ NREM		
	D2R	DREADDs	+	↑ NREM		
		DREADDs	-	↑ wake		
		DREADDs	+	↑ SWS		
A2AR	DREADDs	+	↑ SWS	Oishi et al., 2017		
	Optogenetics	+	↑ SWS			
	DREADDs	-	↓ SWS			
VTA	TH	DREADDs	-	↓ Wake	Eban- Rothschild et al., 2016	
				↑ NREM		
				↑ REM		
		Optogenetics	+	↑ wake		
		DREADDs	-	↓ wake		
		DREADDs	-	↑ NREM		
	Glutamate (Vglut2) GABA (Vgat) GABA (Gad67)	DREADDs	+	↑ wake	Yu et al., 2019	
		DREADDs	+	↑ NREM		
		DREADDs	+	↑ NREM		
		Optogenetics	-	↑ wake		
		Optogenetics	+	↑ NREM		
		DREADDs	-	↑ wake		
VTA → LH VTA → LH	GABA (Gad67)	Optogenetics	+	↑ NREM	Yu et al., 2019	
	GABA (Vgat)	DREADDs	-	↑ wake		
PVT	Glutamate (CAMKIIa)	Optogenetics	+	↑ NREM	Ren et al., 2018	
		DREADDs	-	↓ wake		
		Lesion	-	↓ wake		
		Optogenetics	+	↑ wake		
RMTg	Nonspecific, mostly GABA	DREADDs	+	↑ NREM	Yang et al., 2018	
		DREADDs	-	↓ NREM		
				↑ wake		
		Lesion	-	↓ NREM		
Dorsal raphe	TH	Optogenetics	+	↑ wake	Cho. et al., 2017	
		DREADDs	-	↓ wake		
	SERT	Optogenetics	+	↑ NREM	Oikonomou et al., 2019	
				↓ REM		
		Optogenetics	+	↑ NREM		
				↓ REM		
		Lesion	-	↑ wake		
				↓ NREM		
	POA	PDYN	Optogenetics	+	↓ wake	Chung et al., 2017
					↓ REM	
				↑ NREM		

CAMKIIa, Calcium/calmodulin-dependent protein kinase type II subunit alpha; Gad67, Glutamate decarboxylase; LH, lateral hypothalamus; SERT, serotonin transporter. POA, preoptic area; Vgat, vesicular GABA transporter; Vglut2, vesicular glutamate transporter 2.

subjects significantly increased light NREM sleep and suppresses deep NREM sleep (67% reduction) and REM sleep (25% reduction) (Shaw et al., 2005). Chronic methadone users showed light (poor quality) sleep and, in addition, shorter sleep times and greater fragmentation of sleep (Xiao et al., 2010). A constant and overnight infusion of remifentanyl decreased REM in healthy human volunteers (Bonafide et al., 2008). Patients with chronic noncancer pain with the single nucleotide polymorphism in the μ -opioid receptor (MOR) gene OPRM1 118-GG reported increased sleep disruption and poorer sleep patterns after 3 months of opioid treatment compared with patients with the standard 118-AA genotype (Margarit et al., 2019). Thus, poor sleep is commonly observed with opioid use, and genetics may contribute to the extent of the problem.

Despite the observations that opioids profoundly affect sleep quality, withdrawal from opioid use also has significant effects on sleep architecture. In an early study of heroin users, acute withdrawal resulted in greater sleep/wake transitions and less REM sleep (Howe et al., 1981). In individuals on a methadone maintenance program, acute withdrawal resulted in increased deep NREM sleep and REM sleep for at least 10 weeks (Martin et al., 1973). Whether sleep ever normalizes remains unknown. These experiments show that opioids disrupt sleep in humans, and sleep does not normalize with available medication-assisted therapy for opioid use disorders. Thus, a better understanding of the mechanisms involved in opioid dysregulation of sleep is critical to improve treatments for this aspect of withdrawal.

Preclinical Sleep Studies on the Effects of Opioids

Acute administration of morphine in cats resulted in significant suppression of both NREM and REM sleep with resultant increased wakefulness, particularly in the first few hours after administration (De Andrés and Caballero, 1989). This increased wake (albeit less pronounced) persisted across 2 weeks of daily administration (De Andrés and Caballero, 1989). Interestingly, upon withdrawal after 2 weeks of morphine, the cats, like humans, showed increased NREM and REM sleep (De Andrés and Caballero, 1989). Similarly, morphine administration in rats profoundly suppresses REM sleep acutely and chronically, and withdrawal results in a sustained increase in REM sleep (Khazan and Colasanti, 1972).

Animal studies have brought some insight into the mechanisms of opioid effects on sleep. The medial pontine reticular formation (MPRF) in the brainstem is a region involved in both nociception and REM sleep, and intriguingly, morphine microinjected into the MPRF in the cat suppressed REM sleep (Keifer et al., 1992), which is mediated by activation of MORs in the MPRF (Cronin et al., 1995). Furthermore, direct injection of morphine in the MPRF actually suppressed release of the pro-REM sleep neurotransmitter acetylcholine from the lateral dorsal tegmentum (Lydic et al., 1993). The effects of opioids on sleep are far more complicated and involve more than just the MPRF brainstem effect of morphine suppressing REM sleep because there are additional effects of opioids in several wake-active brain regions as well as sleep-active regions.

Mechanistically, one way acute morphine inhibits slow-wave sleep is via μ -opioid receptors located on GABAergic neurons in the ventrolateral preoptic area (VLPO), a well-known sleep-promoting brain region (Wang et al., 2013). Rats given a subcutaneous injection of morphine showed increased wakefulness and decreased NREM and REM for a 3-hour period after injection (Wang et al., 2013). In brain slices *ex vivo*, morphine hyperpolarized the membrane potential in the VLPO and inhibited firing of these sleep-promoting neurons. This effect was dependent on activation of MORs because the MOR antagonist CTOP prevents morphine-induced hyperpolarization (Wang et al., 2013). However, morphine still partially inhibited the firing of VLPO sleep-promoting neurons even in the presence of CTOP. Full activity was restored in the presence of both morphine and CTOP as well as the κ -opioid receptor (KOR) antagonist, norbinaltorphimine, indicating morphine-induced wakefulness is mediated by both μ - and κ -opioid receptors in the VLPO (Wang et al., 2013). *In vivo*, morphine-induced wakefulness from a 1-mg/kg subcutaneous injection was blocked by CTOP injection into the VLPO, whereas CTOP injection in a vehicle-treated animal did not affect sleep/wakefulness (Wang et al., 2013). μ -Opioid receptor mRNA is expressed in the majority of sleep-promoting neurons in the VLPO, and MOR agonists infused into the VLPO increase wakefulness (Greco et al., 2008). Translational profiling from preoptic neurons followed by RNA sequencing showed that the prodynorphin (PDYN) gene is highly expressed in GABAergic preoptic area neurons (Chung et al., 2017). Prodynorphin encodes for the opioid peptide dynorphin, and optogenetic activation of these PDYN neurons decreases wakefulness and increases NREM (Chung et al., 2017). Local morphine, however, can have sleep/wake effects beyond the VLPO. Injecting morphine into the rostromedial tegmental nucleus (RMTg), also known as the tail of the ventral tegmental area (VTA), hyperpolarizes and inactivates these neurons and decreases NREM and REM while increasing wake in a 3-hour period (Yang et al., 2018).

There is a dearth of research that evaluates the impact of *chronic* opioids or withdrawal on sleep. One of the only studies to do so found that rat self-administration of heroin over a 6-hour period each day reversed the sleep-wake cycle across 14 days of acquisition. Here, rats were placed in the self-administration chambers from 11:00 to 17:00 and in their home cage for the other 18 hours, and EEG/electromyography was recorded continuously. This paradigm resulted in both “low drug takers” and “high drug takers,” but regardless of the amount of drug self-administered, heroin increased time awake during the light cycle and decreased time awake during the dark cycle compared with saline-infused rats (Coffey et al., 2016). During abstinence, wake and NREM returned to baseline circadian rhythms, whereas REM sleep maintained its abnormalities for 3–6 days (Coffey et al., 2016). These studies show a general effect of opioids to reduce or disrupt sleep; however, more studies in this area are needed, especially mechanistic studies assessing the impact of chronic opioid use and extended withdrawal on sleep.

Preclinical Studies on How Sleep Affects Opioid Response

In addition to the effects of opioids on sleep, as described above, findings from several studies demonstrate that disruption of sleep can affect pain sensitivity and response to opioids, leading to a bidirectional feedback loop between sleep and opioids. For example, poor sleep causes many negative symptoms that likely contribute to high rates of opioid relapse, including increased pain sensitivity (Roehrs and Roth, 2005). In humans, sleep deprivation for two consecutive nights lowers thresholds for mechanical pain, and effects are greater for total sleep loss relative to effects of depriving specifically NREM or REM sleep (Onen et al., 2001). Similarly, acute short-term sleep loss (9 hours) in mice resulted in shorter hot-plate withdrawal latencies, which normalize after recovery sleep (Alexandre et al., 2017). Moreover, REM sleep deprivation in the rat across 1 or 2 days lowered the thresholds for mechanical and chemical pain and, importantly, reduced the antinociceptive effect of morphine injected into the periaqueductal gray (Tomim et al., 2016). A pattern of sleep commonly observed in modern societies is chronic short sleep. Chronic short sleep in mice also results in lower pain thresholds in a duration-dependent fashion (Alexandre et al., 2017). Interestingly, heightened pain sensitivity is a consequence of reduced arousal after sleep loss. Specifically, pain thresholds could be normalized with wake-promoting drugs (e.g., caffeine and modafinil), whereas morphine had less effect in countering pain after sleep loss. Together, these findings indicate that the sleep/wake state of an animal affects analgesic properties of opioids (Alexandre et al., 2017). In support of an arousal and pain threshold connection, the hyperalgesia after 96 hours of REM sleep deprivation resulted in loss of tyrosine hydroxylase (TH), the rate-limiting enzyme for dopamine synthesis, and pain threshold could be normalized by administering dopamine systemically prior to testing (Skinner et al., 2011). In contrast, the hyperalgesia after REM sleep deprivation in the rat did not influence regional MOR receptor binding throughout the brain (Nascimento et al., 2007). Poor sleep heightens pain sensitivity (Roehrs et al., 2006), resulting in the requirement for more pain medications. Therefore, improving sleep may be beneficial in reducing tolerance to pain medication, thereby contributing to reductions in opioid use and abuse.

Most studies examining the effect of sleep on opioid use and response to opioids are indirect. Animal studies show that lack of sleep can affect brain activity in regions that modulate opioid response, such as the medial habenula. Sleep fragmentation for 5 days increases the activity of habenula neurons (Ge et al., 2019), and habenula activity is linked to negative affect during morphine withdrawal (Valentinova et al., 2019). In addition, an alpha-3 beta-4 nicotinic receptor antagonist injected into the medial habenula attenuated self-administration of morphine in rats (Glick et al., 2006). Nicotinic receptors in the habenula may indirectly modulate the effects of sleep fragmentation on the extent of negative effects associated with morphine exposure.

The opioid system may also have a role in sleep disruption beyond that resulting from drug use. Disruptions in sleep due to stress are mediated by the KOR, as a 30 mg/kg injection of the KOR antagonist JD1c improved recovery sleep after stress and reversed the expression of the circadian clock gene *mPer2* in the nucleus accumbens (Wells et al., 2017).

Likely Mechanisms of Opioid Effects on Sleep and Areas of Future Studies

Several neurotransmitters and neuromodulators have been shown to be present on wake-activated or sleep-activated neurons, whose activity and signaling mechanisms can also be affected by opioids. Here we summarize recent studies to best determine which neurotransmitters and neuromodulators in various brain regions most likely underlie the effects of opioids on sleep/wake. Schematics of these systems are shown in Figures 1 and 2.

Dopamine. The mesolimbic dopamine pathway plays a key role in mediating rewarding properties of drugs of abuse. Patients with disorders that decrease dopamine levels and function have sleep disturbances (Louter et al., 2012). Chronic morphine enhances the activity of dopamine neurons in the VTA via inhibitory μ -opioid receptors on GABAergic interneurons (Nestler, 2004). VTA soma size decreased, and neuronal excitability increased after two 25-mg subcutaneous morphine pellets that were implanted for 4 days in mice (Mazei-Robison et al., 2011). Decreased volume in the VTA was also observed in postmortem brain slices from human heroin overdose subjects (Mazei-Robison et al., 2011). Dopamine neurons (marked by expression of tyrosine hydroxylase; TH+) in the VTA are more active during wake and REM sleep than NREM sleep (Eban-Rothschild et al., 2016). Optogenetically activating TH+ VTA neurons induces and maintains wakefulness, whereas chemogenetically inhibiting these neurons increases sleep and decreases wakefulness (Eban-Rothschild et al., 2016). In a similar study, designer receptor exclusively activated by designer drugs (DREADD) inhibition of VTA dopamine neurons decreased wakefulness and increased NREM without affecting REM sleep (Yang et al., 2018). In addition, inhibiting dopamine neurons in the substantia nigra decreased wakefulness and increased NREM (Yang et al., 2018). It is of interest that activating dopamine neurons in the VTA causes emergence from anesthesia (Taylor et al., 2016).

TH+ neurons in the VTA may be a locus for the intersection of sleep and reward. The VTA projects to the nucleus accumbens (NAc) via the mesolimbic dopamine pathway. Optogenetic activation of dopamine D1 receptor (D1R)-expressing neurons in the NAc quickly produces wakefulness and extends arousal (Luo et al., 2018). NAc D1R neurons mainly target the substantia nigra, sparsely target the VTA, and can locally inhibit D2R-containing neurons (Luo et al., 2018). Chemogenetic inhibition of nucleus accumbens D2R neurons increased wakefulness and decreased NREM and REM 2 hours after injection of the chemical activator clozapine *N*-oxide injection. Activation of D2R neurons is sleep-promoting, as chemogenetic activation of these receptors increased NREM and decreased wakefulness (Luo et al., 2018). Thus, nucleus accumbens D1R and D2R neurons have opposing effects on sleep/wakefulness. A large subset of dorsal raphe/midbrain neurons are also dopaminergic and project to the VTA (McDevitt et al., 2014), and these neurons express μ -opioid receptors (Li et al., 2016). Optogenetically activating dorsal raphe dopamine neurons is arousing in mice, whereas chemogenetically silencing these neurons induces sleep (Cho et al., 2017). Activity of dopaminergic dorsal raphe neurons from calcium imaging paralleled EEG sleep states in that there was increased activity during wake and decreased

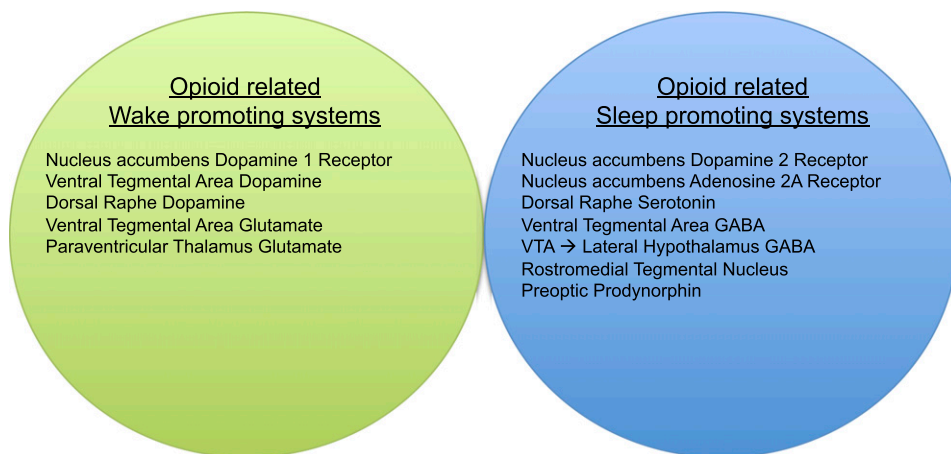


Fig. 1. Opioid-related wake-promoting vs. opioid-related sleep-promoting systems. These regions and cell types have been shown in this review to be related to opioids and affect sleep. The wake-promoting systems are generally activated by opioids, and the sleep-promoting systems are generally inhibited by opioids.

activity during sleep (Cho et al., 2017). These neurons are also activated in response to rewarding stimuli, such as consuming chocolate (Cho et al., 2017). Overall, it appears dopamine neurons across multiple different brain regions contribute to arousal of an animal, and at least components of this dopamine system are engaged by opioid use. Current studies have only examined dopamine's arousal properties immediately after manipulation, and future studies focused on chronic or long-term activation are necessary to determine their role in opioid modulation of sleep/wake states.

Orexin/Hypocretin. Orexin/hypocretin neurons were discovered in the late 1990s (de Lecea et al., 1998; Sakurai et al., 1998) and have a critical role in arousal and sleep/wakefulness (Chemelli et al., 1999; Lin et al., 1999; Tsujino and Sakurai, 2009; Berridge et al., 2010). μ -Opioid receptors are expressed on lateral hypothalamic orexin neurons (Georgescu et al., 2003). Postmortem brains from persons diagnosed with heroin use disorder contained more orexin neurons than control brains (Thannickal et al., 2018). Orexin

neurons originating in the lateral hypothalamus and projecting to the paraventricular nucleus of the thalamus (PVT) were able to mediate wakefulness. Chemogenetic inhibition of these orexin neurons decreased time spent awake and increased time spent in NREM in a 3-hour period (Ren et al., 2018). On the other hand, optogenetic stimulation of this pathway quickly induced wakefulness from NREM and REM (Ren et al., 2018).

In mice, 14 days (but not 7 days) of chronic morphine injections increased the number of immunohistochemically positive orexin cells throughout mouse brain (Thannickal et al., 2018). This 14-day morphine paradigm also increased relative mRNA expression of preprohypocretin and preprodynorphin. Preprohypocretin levels returned to baseline at 2 weeks of withdrawal from morphine (Thannickal et al., 2018). Long-term (60 days) injections at 10, 25, or 50 mg/kg morphine all increase the percentage of orexin cell number. The increase was greatest in the lateral hypothalamus, wherein orexin was synthesized, but morphine also increased

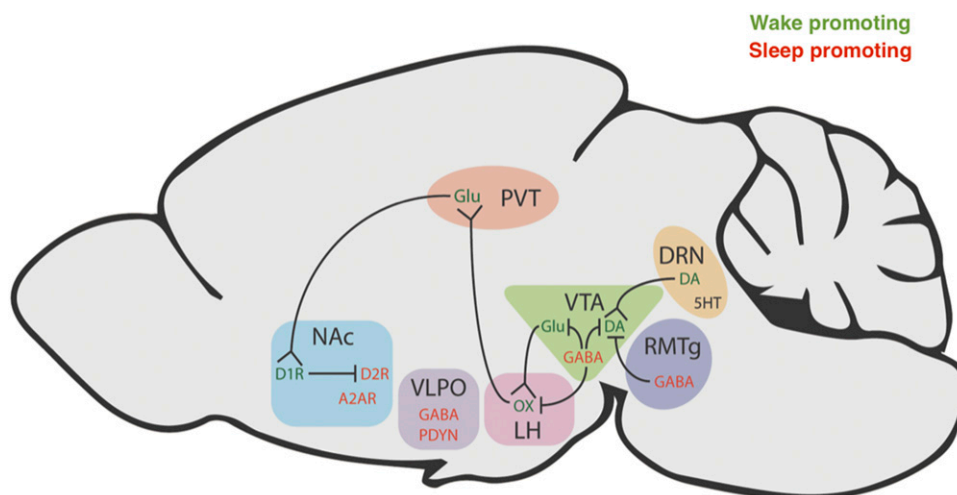


Fig. 2. Neural circuitry of reward/opioid regions involved in sleep/wake. A likely circuit mechanism regulating sleep/wake states that are engaged by opioid use. This figure infers the sleep/wake circuitry in response to opioids. Green indicates wake-promoting systems and red indicates sleep-promoting systems. Many brain regions were tested for neuronal activation or inhibition, but not all were tested for opioid effects on sleep. VLPO and RMTg neurons are sleep-promoting and are inhibited by opioids. This VLPO opioid exposure inhibits sleep-promoting neurons to produce wakefulness, whereas RMTg opioid exposure disinhibits VTA dopamine neurons. Connections between brain regions are drawn for studies in this review that have manipulated projections and examined sleep. For the VLPO, and for adenosine 2A receptor neurons in the nucleus accumbens, experiments examined manipulation of those neurons specifically, and projections to other reward-related brain regions were not established. 5HT, 5-hydroxytryptamine. DA, Dopamine. DRN, Dorsal Raphe Nucleus.

orexin cell number in the medial hypothalamus (Thannickal et al., 2018). Fourteen days of chronic morphine injections kept orexin cell number elevated from control at 2 and 4 weeks of withdrawal, after which time orexin cell number returned to control levels (Thannickal et al., 2018). Interestingly, at 2 weeks after morphine withdrawal, despite increased orexin cell number, the size of each cell was smaller, and this effect went away by the 4th week of withdrawal. In rats, a single 15 mg/kg injection of morphine increased both the action potential discharge in orexin neurons and activity and wakefulness measured by EEG and electromyography (Thannickal et al., 2018). Constitutive orexin knockout mice were narcoleptic (Chemelli et al., 1999). An inducible knockout mouse line was generated recently (Thannickal et al., 2018) to allow maintenance of narcolepsy but more cataplexy than constitutive orexin knockout mice. This more closely resembles narcolepsy in humans and allows for the treatment of cataplectic symptoms. These mice showed decreased orexin cell number compared with wild-type mice, but 14 days of morphine injections could reverse this and return orexin cell number to higher levels while also decreasing cataplexy (Thannickal et al., 2018). This study also showed that human patients with narcolepsy had very few orexin cells postmortem, but patients chronically treated with morphine for pain had increased orexin cell number compared with patients who were narcoleptic but still much less than control patients (Thannickal et al., 2018). Thus, data from immunohistochemistry, electrophysiology, and molecular analysis demonstrate an interaction of orexin and morphine resulting in increased wakefulness.

Orexin neurons increase their activity and peptide discharge in wakefulness compared with sleep (Thannickal et al., 2018) and express the immediate early gene *c-Fos* in response to morphine (Georgescu et al., 2003; Harris et al., 2007). Orexin neurons promote arousal when optogenetically activated (Adamantidis et al., 2007) and project to reward processing regions, such as the ventral tegmental area (Marcus et al., 2001). These projections to the VTA affect synaptic plasticity (Baimel and Borgland, 2015). Suvorexant is a dual orexin receptor antagonist the Food and Drug Administration–approved for the treatment of insomnia, but it is currently in a clinical trial for addiction (ClinicalTrials.gov identifier: NCT03412591). Thus, blocking orexin neurons with pharmacological therapeutics could be an avenue to treat morphine-induced sleep disruption.

Serotonin. The role of serotonin in sleep has been controversial, with early studies showing a sleep-promoting role (Mouret et al., 1968; Jouvet, 1969, 1972) and later experiments showing serotonin as a wake-activated neurotransmitter (Ursin, 2002; Lopez-Rodriguez et al., 2003; Zant et al., 2011). Serotonergic projections to the VTA excite VTA dopamine neurons (Wang et al., 2019), and rostral VTA neurons transmit GABA to disinhibit MOR-expressing serotonin cells in the dorsal raphe (Li et al., 2019). Studies examining direct serotonergic modulation of morphine-induced sleep/wake behaviors have not been done. However, a number of indirect studies show serotonin effects on sleep as well as serotonin effects on negative affect during withdrawal may contribute to opioid-mediated alterations in sleep/wake states.

Dorsal raphe serotonin neuron activity tracked with sleep/wake states in mice as fiber photometry fluorescence showed increased activity in dorsal raphe serotonin neurons in wake

states and decreased activity during sleep (Oikonomou et al., 2019). Lesioning dorsal raphe serotonergic cells in mice increased wakefulness and decreased NREM and REM, and optogenetic stimulation of these neurons increased wake probability with burst firing but decreased wake probability with tonic firing (Oikonomou et al., 2019). It is of interest that optogenetic activation of serotonin terminals projecting from the dorsal raphe to the VTA was rewarding. Activation of serotonergic dorsal raphe to VTA projections increased time spent in a CPP chamber paired with this stimulation (Wang et al., 2019). Chemogenetically activating dorsal raphe serotonin neurons shifts circadian timing of active/rest cycles and decreases probability of active states in the dark cycle while increasing the probability of active states in the light cycle (Urban et al., 2016).

Negative affect and sociability deficits are associated with protracted opiate withdrawal and are mediated by serotonin signaling. At 4 weeks of morphine withdrawal, serotonin turnover specifically in the dorsal raphe was increased, and the selective serotonin reuptake inhibitor fluoxetine reversed sociability deficits and immobility in the tail suspension test that resulted from morphine withdrawal (Goeldner et al., 2011). Tumor necrosis factor- α signaling from the lateral habenula projecting to the dorsal raphe was responsible for sociability deficits due to morphine withdrawal (Valentinova et al., 2019). Lastly, serotonin neurons can also project to orexin neurons and appear to regulate sleep architecture via 5-hydroxytryptamine 1a receptors (Saito et al., 2018). In this study, mice lacking 5-hydroxytryptamine 1a receptors exclusively in orexin neurons showed decreased wakefulness and increased NREM at the end of the dark cycle and also increased REM at the beginning of the light cycle (Saito et al., 2018). Further mechanistic studies are necessary to confirm the interaction of opioids in the serotonergic raphe system with sleep/wake and withdrawal behaviors.

Adenosine. Caffeine, the most widely used drug to increase alertness, acts by blocking adenosine receptors (Nehlig et al., 1992). Morphine, fentanyl, and buprenorphine decreased adenosine levels within the sleep-regulating pontine reticular formation and substantia innominata in rats (Nelson et al., 2009; Gauthier et al., 2011), whereas injecting adenosine into the pontine reticular formation promoted sleep in rats (Coleman et al., 2006). Adenosine also acted at adenosine 2A receptors (A2ARs) in the VLPO to increase sleep (Scammell et al., 2001). Therefore, adenosine may be a critical regulator at the interface between disrupted sleep and opioid exposure [for review see Moore and Kelz (2009)]. Optogenetic and chemogenetic activation of A2AR-expressing neurons in the NAc core (but not shell) induces slow-wave sleep (Oishi et al., 2017). Inhibiting these NAc core A2AR neurons is wake-promoting and decreases slow-wave sleep. In addition, these neurons show less *c-FOS* expression in response to motivational stimuli (Oishi et al., 2017). Motivational stimuli, such as toys and chocolate, also decrease slow-wave sleep and *c-FOS* expression in these NAc A2AR neurons that project to the ventral pallidum (Oishi et al., 2017).

Glutamate. Glutamate neurons in the paraventricular thalamus mediate wakefulness. Multichannel electrophysiology showed that PVT glutamate neurons have a higher firing rate during wake compared with NREM and REM. Chemogenetic inhibition of these neurons decreases wakefulness and increases NREM and REM (Ren et al., 2018).

Optogenetic stimulation of PVT glutamate neurons increases the probability of wake while decreasing the probability of NREM and decreases latency to wake both from sleeping and from isoflurane-induced anesthesia (Ren et al., 2018). PVT glutamate projections to the NAc are responsible for controlling wakefulness; however, those that project to the medial prefrontal cortex or insula do not have any impact on wake states (Ren et al., 2018). PVT neurons that project to the NAc also elicit c-FOS activation upon spontaneous withdrawal from morphine, and optogenetic inhibition of this pathway blocks somatic signs of opioid withdrawal (Zhu et al., 2016). Optogenetic stimulation of PVT glutamate terminals in the nucleus accumbens decreases latency to wake from both NREM and REM, and chemogenetic inhibition of this pathway decreases time spent in wake and increases time spent in NREM (Ren et al., 2018).

In addition to dopamine neurons, the VTA also contains GABA and glutamatergic neurons that were discovered via an unbiased screen to control arousal (Yu et al., 2019). These are mostly separate from VTA dopamine neurons—about 25% of glutamatergic neurons were TH+, indicating only a small percentage of neurons have colocalized glutamate and dopamine, and the wakefulness phenotype driven by glutamate was maintained in the presence of dopamine antagonists (Yu et al., 2019). Glutamatergic neurons in the VTA promote wakefulness, whereas GABAergic neurons in the VTA promote sleep (Yu et al., 2019). Selective chemogenetic or optogenetic stimulation of glutamatergic cells in the VTA produced wakefulness exclusively for a 5-hour period. Calcium imaging showed that these neurons are more active during wake and REM than they are during NREM (Yu et al., 2019). Glutamate in these brain regions is a newly identified mechanism for regulating sleep/wake that is seen in opioid-responsive brain regions; however, direct effects of opioids on glutamate signaling in the VTA and PVT have not been tested.

GABA. GABAergic neurons in the VTA have a sleep-promoting role, as lesioning VTA GABA neurons elicits wakefulness (Yu et al., 2019). DREADDs stimulation of VTA GABA neurons decreases wake and increases NREM and REM, whereas DREADDs or optogenetic inhibition oppositely increase wake and decrease NREM and REM (Chowdhury et al., 2019; Yu et al., 2019). The mechanism proposed for VTA GABA neurons modulating wakefulness involves inhibiting nearby VTA dopamine and VTA glutamate neurons that both produce wakefulness (Yu et al., 2019). VTA GABA stimulation is behaviorally aversive and inhibits VTA dopamine (Yu et al., 2019). The RMTg is a sleep-promoting region that contains μ -opioid receptors (Matsui et al., 2014). This nucleus receives input from the lateral habenula and is generally considered to be an inhibitory check on the midbrain dopamine system because RMTg GABA neurons project to and inhibit VTA dopamine neurons (Yang et al., 2018). Because VTA dopamine neurons promote wakefulness and RMTg GABA neurons inhibit VTA dopamine neurons, it follows that RMTg GABA neurons are sleep-promoting. Indeed, chemogenetic activation of RMTg neurons increases NREM sleep (Yang et al., 2018). This study used a virus to drive excitatory DREADDs expression in the RMTg, and although it was not targeted with a cell type-specific promoter, immunohistochemistry showed that nearly all (87%) of the virally infected neurons were GABAergic (Yang et al., 2018). RMTg DREADDs stimulation increased NREM and decreased wakefulness

and REM in 7 hours after clozapine *N*-oxide injection. Lesioning RMTg neurons with ibotenic acid increased wake and decreased NREM and REM in a 12-hour period at 8 and 16 days after the lesion (Yang et al., 2018). Optogenetic stimulation of RMTg terminals in the VTA was sufficient to inhibit VTA dopamine neurons from firing (Yang et al., 2018). Furthermore, DREADDs inhibition of VTA dopamine neurons decreases wakefulness and increases NREM without affecting REM sleep (Yang et al., 2018). Thus, the mechanism of opioids modulating wakefulness appears to be via sleep-promoting RMTg GABA neurons influencing midbrain dopamine neurons.

Conclusion

As described above, multiple groups of “reward” neurons impact sleep/wakefulness and are influenced by opioid receptor activation. Opioids induce both activation of wake-promoting systems and inhibition of sleep-promoting systems (Fig. 1). Additionally, wake-promoting and sleep-promoting circuits are mutually inhibitory. Thus, inhibiting or exciting any one of the regions will impact the others. Specifically, studies of optogenetic and chemogenetic manipulation of reward neurons that impact sleep/wakefulness provide a likely circuit that is engaged to promote wakefulness and reward upon opioid use by facilitating activation of wake-promoting systems and inhibition of sleep-promoting systems (Figs. 1 and 2). Based on these studies, a likely circuit involves opioid-mediated excitation of VTA dopamine neurons that are wake-promoting and projecting to the nucleus accumbens D1 receptor neurons. The sleep-promoting D2 receptor neurons and adenosine 2A receptor neurons in the nucleus accumbens are inhibited. Lateral hypothalamus orexin neurons, which are wake-promoting, are excited by morphine and project to the paraventricular thalamus. The PVT may then send glutamatergic signals to the nucleus accumbens. Dorsal raphe dopamine neurons are influenced by opioids and project to VTA dopamine neurons. VTA GABA neurons as well as RMTg GABA neurons inhibit these midbrain dopamine neurons, whereas VTA glutamate neurons promote a role for wakefulness (Fig. 2). Clearly the effects of opioids on sleep are dose-, region-, and cell type-dependent, as high doses of opioids are sedative. Although some effort has been devoted to uncovering this, little is understood about the biology of opioid effects on sleep. The studies reviewed here have identified mechanisms associated with sleep, opioid response, and reward. More critical testing of key regions involved in opiate effects on sleep is much needed. For example, future studies could delete μ -opioid receptors exclusively in the dopaminergic VTA or GABAergic VLPO neurons and examine whether the acute, chronic, and withdrawal effects of opioid administration are abolished. Optogenetic studies have been able to show immediate arousal from sleep states when reward-related neurons are activated, and chemogenetic studies have been able to extend arousal when animals would typically go back into a sleep state. However, to uncover the mechanism of chronic opioids on sleep and the effects of disrupted sleep on opioid use, optogenetics may be limiting by activation or inhibition on too short of a timescale. Future experiments could focus on chemogenetic manipulation of VTA neurons during chronic opioid use to evaluate the effects of these midbrain dopamine neurons on sleep and reward. The

dorsal raphe may be implicated in opioid withdrawal and sleep, and chemogenetic manipulation in the dorsal raphe during withdrawal could be used to influence sleep and withdrawal-related behaviors. Finally, are opioid effects on sleep ever fully reversed, and if not, by what mechanisms is sleep persistently disrupted?

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