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Title Page

Bidirectional relationship between opioids and disrupted sleep: putative mechanisms

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Running Title Page

a) Running title

Opioids and disrupted sleep: putative mechanisms

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d) Nonstandard abbreviations

α3β4= alpha-3 beta-4 nicotinic receptor
A2AR= Adenosine 2A Receptor
CNO= Clozapine N-Oxide
CTOP= D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH2,
D1R= Dopamine D1 Receptor
D2R= Dopamine D2 Receptor
DR= Dorsal Raphe
DREADDs= Designer Receptors Exclusively Activated by Designer Drugs

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EEG= electroencephalography
EMG= electromyography
KOR= Kappa Opioid Receptor
LH= Lateral Hypothalamus
MOR= Mu Opioid Receptor
MPRF= Medial Pontine Reticular Formation
NAc= Nucleus Accumbens
norBNI= Norbinaltorphimine
NREM= Non Rapid Eye Movement
OUD= Opioid Use Disorder
PDYN= Prodynorphin
PVT= Paraventricular Nucleus of the Thalamus
qPCR= quantitative Polymerase Chain Reaction
REM= Rapid Eye Movement
RMTg= Rostromedial Tegmental Nucleus
SWS= Slow Wave Sleep
TH+= Tyrosine Hydroxylase positive
TNFα = Tumor Necrosis Factor alpha
VLPO= Ventrolateral Preoptic Area
VTA= Ventral Tegmental Area
5HT= 5-hydroxytryptamine

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Abstract

Millions of Americans suffer from opiate use disorder, and over a hundred 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. While the physical and psychological 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 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

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this relationship is not understood. This review compiles recent studies in this area that fill this gap in knowledge.

1. Introduction

Every day in the United States, there are over 130 deaths due to opioid overdose (US Department of Health and Human Services, 2018). 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 (OUD) (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 (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, could also contribute to high relapse rates. There may be a common vicious cycle of opioid use to induce sleep disturbances, increasing further usage of opioids. For example, poor sleep can be a risk factor for drug use, especially in adolescents.

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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 *et al.*, 2018). Understanding the biology linking sleep and opioids as well as targeting therapeutics to improve sleep during opioid withdrawal is 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.

2. Effects of opioids on human sleep

One of the earliest descriptions of opioid effects on sleep in humans involved 4 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 3 or 7 consecutive nights (Lewis *et al.*, 1970). Acute administration of heroin resulted in suppression of

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rapid-eye-movement sleep (REM sleep) by 50% with smaller reductions following consecutive nights of heroin administration. Upon withdrawal, REM sleep rebound was present and persisted for days. Additionally, heroin administration suppressed deep non-rapid-eye-movement (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 sulphate to healthy young adult subjects significantly increases light NREM sleep and suppresses deep NREM sleep (67% reduction) and REM sleep (25% reduction) (Shaw *et al.*, 2005). Chronic methadone users show 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 remifentanil decreases REM in healthy human volunteers (Bonafide *et al.*, 2008). Chronic noncancer pain patients with the single nucleotide polymorphism in the mu opioid receptor gene OPRM1 118-GG report increased sleep disruption and poorer sleep patterns after 3 months of opioid treatment compared to 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

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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 results 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.

3. **Preclinical sleep studies on the effects of opioids**

Acute administration of morphine in cats results 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) persists across 2 weeks of daily administration (De Andrés and Caballero, 1989). Interestingly, upon withdrawal after two 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 increased 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

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morphine microinjected into the MPRF in the cat suppresses REM sleep (Keifer *et al.*, 1992) which is mediated by activation of mu opioid receptors (MORs) in the MPRF (Cronin *et al.*, 1995). Further, direct injection of morphine in the MPRF actually suppresses 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, as 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 mu 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 show increased wakefulness and decreased NREM and REM for a 3h period after injection (Wang *et al.*, 2013). In brain slices *ex vivo*, morphine hyperpolarizes the membrane potential in the VLPO and inhibits firing of these sleep-promoting neurons. This effect is dependent on activation of MORs as the MOR- antagonist CTOP prevents morphine-induced hyperpolarization (Wang *et al.*, 2013). However, morphine still partially inhibits the firing of VLPO sleep promoting neurons even in the presence of CTOP. Full activity is restored in the presence of both morphine and CTOP, and the kappa opioid receptor (KOR) antagonist nor-BNI, indicating morphine induced wakefulness is mediated by both mu and kappa opioid receptors in the VLPO (Wang *et al.*, 2013). *In vivo*, morphine-induced wakefulness from a 1mg/kg subcutaneous injection was blocked by CTOP injection into the VLPO, while

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CTOP injection in a vehicle treated animal did not affect sleep/wakefulness (Wang *et al.*, 2013). Mu 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 shows that the prodynorphin gene (PDYN) is highly expressed in GABAergic preoptic area neurons (Chung *et al.*, 2017). Prodynorphin encodes for the opioid peptide dynorphin, and optogenetic activation of these prodynorphin (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 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 reverses the sleep-wake cycle across 14 days of acquisition. Here, rats were placed in the self-administration chambers from 11:00-17:00 and in their home cage for the other 18 hours, and EEG/EMG 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 increases time awake during the light cycle and decreases time awake during the dark cycle compared to saline infused rats (Coffey *et al.*, 2016). During abstinence, wake and NREM return to baseline

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circadian rhythms, while REM sleep maintains 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.

4. 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 (9hours) in mice results in shorter hot plate withdrawal latencies, which normalize after recovery sleep (Alexandre *et al.*, 2017). Moreover, REM sleep deprivation in the rat across one or two days lowers the thresholds for mechanical and chemical pain, and importantly reduces the anti-nociceptive effect of morphine injected into the periacquaductal grey (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

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reduced arousal after sleep loss. Specifically, pain thresholds can be normalized with wake-promoting drugs, e.g., caffeine and modafinil, while 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 following 96 hrs of REM sleep deprivation results in loss of tyrosine hydroxylase (TH)- the rate limiting enzyme for dopamine synthesis, and pain threshold can be normalized by administering dopamine systemically prior to testing (Skinner *et al.*, 2011). In contrast, the hyperalgesia following REM sleep deprivation in the rat does 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 α3β4 nicotinic receptor antagonist injected into the medial habenula attenuates self-administration of morphine in rats (Glick *et al.*, 2006). Nicotinic receptors in the habenula may

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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 30mg/kg injection of the KOR antagonist JDTic improves recovery sleep after stress and reverses the expression of the circadian clock gene mPer2 in the nucleus accumbens (Wells *et al.*, 2017).

5. Likely mechanisms of opioid effects on sleep and areas of future studies

Several neurotransmitters and neuromodulators have been shown 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 (Figure 1 and 2).

a. 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 ventral tegmental area (VTA) via inhibitory mu opioid receptors on GABAergic interneurons (Nestler, 2004). VTA soma size decreases and neuronal excitability increases following two 25mg subcutaneous morphine pellets that were

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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, while chemogenetically inhibiting these neurons increases sleep and decreases wakefulness (Eban-Rothschild *et al.*, 2016). In a similar study, DREADD inhibition of VTA dopamine neurons decreases wakefulness and increases NREM without affecting REM sleep (Yang *et al.*, 2018). In addition, inhibiting dopamine neurons in the substantia nigra decreases wakefulness and increases NREM (Yang *et al.*, 2018). Of interest, activating dopamine neurons in the VTA even 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 produce wakefulness and extend 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 increases wakefulness and decreases NREM and REM 2 hours after injection of the chemical activator Clozapine-N-Oxide (CNO) 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 (DR)/midbrain neurons are also dopaminergic and project to the VTA (Mcdevitt *et al.*, 2014) and these neurons express mu opioid receptors (Li *et al.*, 2016). Optogenetically activating dorsal raphe dopamine neurons is arousing in mice, while chemogenetically silencing these neurons induces sleep (Cho *et al.*, 2017). Activity of dopaminergic dorsal raphe neurons from calcium imaging parallels EEG sleep states in that there is increased activity during wake and decreased 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.

b. 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). Mu opioid receptors are expressed on lateral hypothalamic orexin neurons (Georgescu *et al.*, 2003). Postmortem brains from persons diagnosed with heroin use disorder contain more orexin neurons than

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control brains (Thannickal *et al.*, 2018). Orexin neurons originating in the lateral hypothalamus and projecting to the paraventricular nucleus of the thalamus (PVT) are able to mediate wakefulness. Chemogenetic inhibition of these orexin neurons decreases time spent awake and increases time spent in NREM in a 3 hour period (Ren *et al.*, 2018). On the other hand, optogenetic stimulation of this pathway quickly induces wakefulness from NREM and REM (Ren *et al.*, 2018).

In mice, 14 days (but not 7 days) of chronic morphine injections increase the number of immunohistochemically positive orexin cells throughout mouse brain (Thannickal *et al.*, 2018). This 14-day morphine paradigm also increases 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 50mg/kg morphine all increase the percentage of orexin cell number. The increase was greatest in the lateral hypothalamus, where orexin is synthesized, but morphine also increased orexin cell number in the medial hypothalamus (Thannickal *et al.*, 2018). 14 days of chronic morphine injections keeps orexin cell number elevated from control at 2 and 4 weeks of withdrawal, after which time orexin cell number returns to control levels (Thannickal *et al.*, 2018). Interestingly, at 2 weeks after morphine withdrawal, despite increased orexin cell number, the size of each cell is smaller and this effect goes away by the 4th week of withdrawal. In rats, a single 15mg/kg injection of morphine increases both the action potential discharge in orexin neurons and activity and wakefulness measured by EEG and EMG (Thannickal *et al.*, 2018). Constitutive orexin

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knockout mice are narcoleptic (Chemelli *et al.*, 1999). An inducible knock-out 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 show decreased orexin cell number compared to wildtype mice, but 14 days of morphine injections can 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 to narcoleptic patients, 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, FDA approved for the treatment of insomnia, but is currently in a clinical trial for addiction (ClinicalTrials.gov Identifier:

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NCT03412591). Thus, blocking orexin neurons with pharmacological therapeutics could be an avenue to treat morphine induced sleep disruption.

c. 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 tracks with sleep/wake states in mice as fiber photometry fluorescence shows 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 increases wakefulness and decreases NREM and REM, and optogenetic stimulation of these neurons increases wake probability with burst firing but decreases wake probability with tonic firing (Oikonomou *et al.*, 2019). Of interest, optogenetic activation of serotonin terminals projecting from the dorsal raphe to the VTA is rewarding. Activation of serotonergic DR to VTA projections increases

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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.*, 2015).

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 is increased, and the SSRI Fluoxetine reverses sociability deficits and immobility in the tail suspension test that resulted from morphine withdrawal (Goeldner *et al.*, 2011). TNFα signaling from the lateral habenula projecting to the dorsal raphe is 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 5HT1a receptors (Edward Brown *et al.*, 2018). In this study, mice lacking 5HT1a receptors exclusively in orexin neurons show decreased wakefulness and increased NREM at the end of the dark cycle, and also increased REM at the beginning of the light cycle (Edward Brown *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.

d. Adenosine

Caffeine, the most widely used drug to increase alertness, acts by blocking adenosine receptors (Nehlig *et al.*, 1992). Morphine, fentanyl, and

buprenorphine decrease adenosine levels within the sleep-regulating pontine reticular formation and substantia innominata in rats (Nelson *et al.*, 2009; Gauthier *et al.*, 2011), while injecting adenosine into the pontine reticular formation promotes sleep in rats (Coleman *et al.*, 2006). Adenosine also acts at A2AR receptors 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 adenosine 2a receptor (A2AR) expressing neurons in the nucleus accumbens (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).

e. Glutamate

Glutamate neurons in the paraventricular thalamus mediate wakefulness. Multichannel electrophysiology shows 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

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et al., 2018). PVT glutamate projections to the NAc are responsible for controlling wakefulness, however those that project to the mPFC 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 positive, 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 while 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

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responsive brain regions, however direct effects of opioids on glutamate signaling in the VTA and PVT have not been tested.

f. 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, while DREADDs or optogenetic inhibition oppositely increases wake and decreases 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 Rostromedial Tegmental Nucleus (RMTg) is a sleep promoting region that contains mu 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, as 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 while 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

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DREADDs stimulation increases NREM and decreases wakefulness and REM in 7 hours after CNO injection. Lesioning RMTg neurons with ibotenic acid increases wake and decreases NREM and REM in a 12 hour period at 8 days and 16 days after the lesion (Yang *et al.*, 2018). Optogenetic stimulation of RMTg terminals in the VTA is 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 (Figure 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 (Figures 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, while VTA glutamate neurons promote a role for wakefulness (Figure 2). Clearly the effects of opioids on sleep are dose, region, and cell-type dependent, as high doses of opioids are sedative. While 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 are much needed. For example, future studies could delete mu 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

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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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Table 1. Manipulation of opioid/reward related neurons and their effects on sleep

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	

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		DREADDs	−	↑ wake	
	A2AR	DREADDs	+	↑ SWS	Oishi et. al. 2017
		Optogenetics	+	↑ SWS	
		DREADDs	−	↓ SWS	
VTA	TH	DREADDs	−	↓ Wake ↑ NREM ↑ REM	Eban-Rothschild et. al. 2016
		Optogenetics	+	↑ wake	
		DREADDs	−	↓ wake ↑ NREM	Yang et. al. 2018
	Glutamate (Vglut2)	DREADDs	+	↑ wake	Yu et. al. 2019
	GABA (Vgat)	DREADDs	+	↑ NREM	
	GABA (Gad67)	DREADDs	+	↑ NREM	Chowdhury et. al. 2019
		Optogenetics	−	↑ wake	
VTA → LH	GABA (Gad67)	Optogenetics	+	↑ NREM	
VTA → LH	GABA (Vgat)	DREADDs	−	↑ wake	Yu et. al. 2019
		Optogenetics	+	↑ NREM	

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PVT	Glutamate (CAMKIIa)	DREADDs	−	↓ wake	Ren et. al. 2018
		Lesion	−	↓ wake	
		Optogenetics	+	↑ wake	
RMTg	Nonspecific, mostly GABA	DREADDs	+	↑ NREM	Yang et. al. 2018
		DREADDs	−	↓ NREM ↑ wake	
		Lesion	−	↓ NREM ↓ REM	
Dorsal Raphe	TH	Optogenetics	+	↑ wake	Cho. et. al. 2017
		DREADDs	−	↓ wake	
	SERT	Optogenetics	+ (burst)	↓ NREM ↓ REM	Oikonomou et. al. 2019
		Optogenetics	+ (tonic)	↑ NREM ↓ REM	
		Lesion	−	↑ wake ↓ NREM ↓ REM	

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POA	PDYN	Optogenetics	+	↓ wake ↑ NREM	Chung et. al. 2017

Table 1. A table of recent studies that manipulate neurons in reward related brain region 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.

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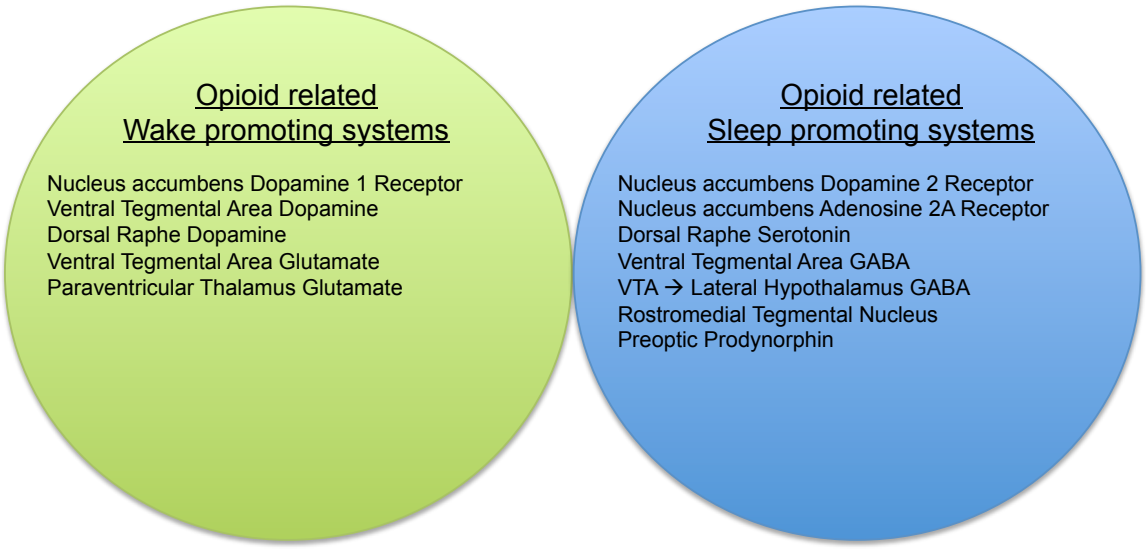

Figure 1- Opioid related wake promoting versus opioid related sleep promoting systems. These regions and cell types have been shown in this review to be related to opioids and affect sleep. The wake promoting systems are generally activated by opioids and the sleep promoting systems are generally inhibited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

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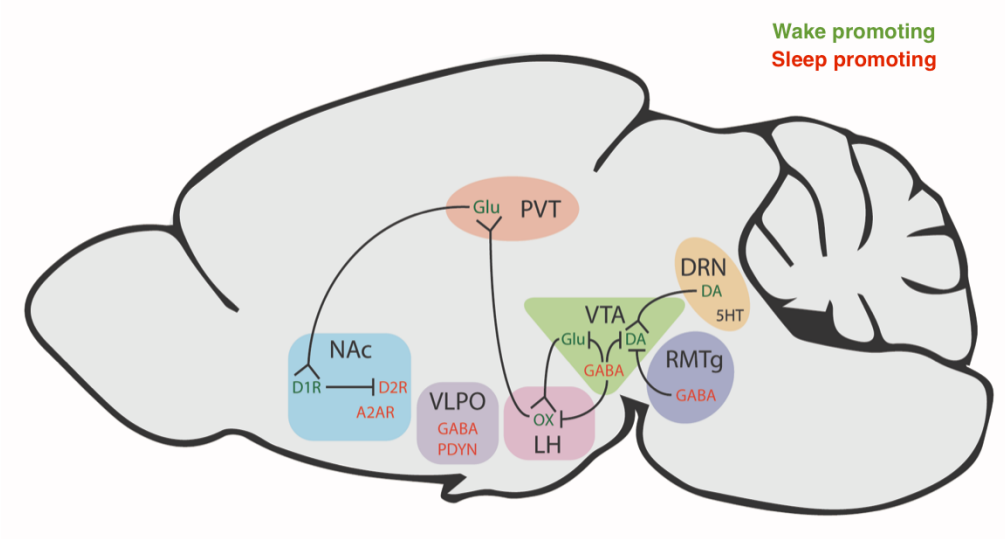

Figure 2: Neural circuitry of reward/opioid regions involved in sleep/wake. A likely circuit mechanism regulating sleep/wake states that are engaged by opioid use. This figure infers the sleep/wake circuitry in response to opioids. Green indicates wake-promoting systems and red indicates sleep-promoting 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, while 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.

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