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Sites of Action of Sleep and Wake Drugs: Insights from Model Organisms

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Abstract

Small molecules have been used since antiquity to regulate our sleep. Despite the explosion of diverse drugs to treat problems of too much or too little sleep, the detailed mechanisms of action and especially the neuronal targets by which these compounds alter human behavioural states are not well understood. Research efforts in model systems such as mouse, zebrafish, and fruit fly are combining conditional genetics and optogenetics with pharmacology to map the effects of sleep-promoting drugs onto neural circuits. Recent studies raise the possibility that many small molecules alter sleep and wake via specific sets of critical neurons rather than through the global modulation of multiple brain targets. These findings also uncover novel brain areas as sleep/wake regulators and indicate that the development of circuit-selective drugs might alleviate sleep disorders with fewer side effects.

Introduction

Sleep potions and arousing elixirs have featured in both legend and practice since ancient times. While dominated for centuries by only a few compounds, the modern medical arsenal features an increasing variety of drugs for the treatment of sleep disorders such as insomnia, hypersomnia, and narcolepsy. These disorders affect millions of people each year, and in the United States alone, more than two billion dollars are spent on sleep aids. Despite the widespread use of sleep- and wake-promoting drugs, much remains unknown about the basic mechanisms and sites of action by which these drugs work [1].

There are many reasons for the gap in understanding. Sleep and arousal are highly complex brain states, involving many different neural circuits and neurotransmitter systems. In some cases, these sleep-promoting drugs have significant affinity for multiple protein targets, each of which may have unique pharmacological properties, anatomical sites of action, and functional outcomes. Furthermore, the molecular targets are expressed in neurons

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throughout the brain, raising questions about whether specific or global neuronal populations are targeted to modulate sleep. Finally, a large constellation of drugs can cause drowsiness but do not necessarily recapitulate normal sleep [2*].

Recent conceptual and experimental advances in model organisms are providing new insights into the neural and molecular mechanisms for sleep- and wake-promoting drugs. In mice, conditional knockout technology, deliverable by stereotactic injection of viruses in adult brains, provides both spatial and temporal control of sleep gene function. Moreover, there is an increased recognition of the conservation of sleep genetics and pharmacology in less complex model systems including zebrafish [3**, 4] and fruit flies [5]. This has allowed experiments to link sleep- and wake-altering drugs to discrete neural circuits. In this review, we discuss how research in these model systems provides new insights into the molecular and neuronal targets (Figure 1) of major classes of sleep- and wake-regulating drugs (Table 1).

$GABA_A$ Receptor Agonists

The hypnotic benzodiazepines and Z-drugs (i.e. zolpidem, eszopiclone, and zaleplon, also known in the US by the brand names Ambien, Lunesta, and Sonata, respectively) are popular sleep drugs. They enhance signalling of the brain's major inhibitory neurotransmitter, γ-aminobutyric acid (GABA), via the type A receptor. $GABA_A$ receptors are heteropentameric ligand-gated ion channels typically composed of two α (with six possible isoforms), two β (3 isoforms), and one γ (3 isoforms) subunits [6*]. Benzodiazepines and Z-drugs potentiate $GABA_A$ receptor signalling via a modulatory binding site found in α subunits, with Z-drugs selective for the α1 subtype. The diversity of receptor subunit composition coupled with widespread brain expression has raised the questions of how and where the action of these drugs on $GABA_A$ receptors induces sleep.

Genetic replacement experiments in mice, in which the various alpha subunits are genetically replaced with versions that lack the benzodiazepine binding site, have begun to dissect which subunits are important for the major drug-induced phenotypes, including sedation, anxiety, and addiction. These experiments reveal that the α1 subunit is critical for sedation and the α5 subunit for developing benzodiazepine tolerance [7, 8]. These swap experiments reveal that at least some subsets of the myriad drug effects require different GABA-receptor subunits. Similarly, correlates of addiction were shown to depend on functional α1 binding sites in GABAergic neurons that modulate dopamine signalling in the ventral tegmental area [9]. That both the sedative and addictive properties of benzodiazepines are linked to the α1 subunit highlights that these drugs, including the α1 selective Z-drugs, do not act exclusively and specifically as hypnotics.

Does the hypnotic action of $GABA_A$ receptor drugs depend on global modulation or regulation of only a critical set of neurons? According to the flip-flop model of sleep-wake regulation, wake-promoting neurons, including the histaminergic tuberomammillary nucleus (TMN), the noradrenergic locus coeruleus (LC), and the serotonergic neurons of the dorsal raphe sit in a mutually inhibitory switch with sleep-active GABAergic neurons of the ventrolateral preoptic area (VLPO; Figure 1A). During sleep, GABA release from the VLPO is thought to inhibit these wake-promoting centers via GABA-receptors, making these areas prime candidates for the sedative action of GABAergic drugs. However, the importance of these areas in drug activity remains unclear. For example, the genetic elimination of GABA signalling specifically from the wake-inducing TMN histaminergic neurons has no effect on mouse sleep or on sensitivity to GABAergic drugs [10*]. Instead, viral-mediated ablation of the α1 $GABA_A$ subunits in the adult mouse amygdala—a brain area understudied in sleep research—abolishes the motor and sedative effects of zolpidem [11]. GABA agonists also

do not clearly act upstream to disinhibit the GABA neurons of the VLPO, as Z-drugs such as eszopiclone do not enhance immediate early gene expression in the VLPO [12]. Intriguingly, the sedative effects of a different drug class, the anaesthetic isoflurane, appear due to the direct activation of the VLPO GABAergic neurons in mammals (Figure1A) and sleep-promoting neurons in the *Drosophila* fan-shaped body (Figure 1B), demonstrating that direct drug modulation of sleep-promoting circuits can in principle account for sedative properties of some small molecules [13**, 14].

While the neuronal specificity of GABA agonists in mammals remains unresolved, insights from *Drosophila* are framing how GABA-receptor-mediated inhibition of only a few neurons can account for drugs' hypnotic properties. A *Drosophila* mutant with a slow-to-desensitize $GABA_A$ receptor (Resistant to dieldrein; Rdl) decreases fly sleep latency, while the drug carbamazepine, which enhances channel desensitization, increases latency [15]. These sleep-modulating effects critically depend on functional $GABA_A$ receptors in a set of wake-promoting circadian clock neurons, the ventral lateral neurons (Figure 1B) [16**, 17**]. Thus, despite widespread GABA receptor expression in flies, pharmacological actions on select wake-promoting cells underlie the sedative effects of GABA drugs and suggest that these wake-promoting centers lie downstream of sleep-promoting GABAergic circuits, as in mammals. Additional selective replacement studies are needed to determine whether GABA-receptor agonists also act on specific circuits in mammals. Zebrafish larvae are another model suitable for studying the role of GABA signalling in sleep, as GABA-A receptor agonists, including steroidal modulators of $GABA_A$ signalling such as alfadolone, enhance zebrafish sleep, while $GABA_A$ receptor antagonists inhibit sleep [3**].

Histamine H1 and H3 receptor antagonists

Low dose doxepin (Silenor) is a histamine H1 receptor antagonist approved for insomnia treatment in 2010 [18]. Anti-histamine receptor drugs that cross the blood brain barrier have been used as over-the-counter sleep aids for decades, although the complex pharmacological profiles of first generation anti-histamines such as diphenhydramine and chlorpheniramine has obscured the precise mechanisms of action for causing drowsiness (reviewed in [19*]).

In mammals, histamine is predominately made by the TMN (Figure 1A) and signals through four histamine receptors (H1–H4), which are widely expressed in many neuronal types in the brain, including the cerebral cortex and many wake-promoting areas such as the LC and the basal forebrain [19*]. Numerous pharmacological and genetic studies implicate the H1 receptor as the major wake-promoting histamine target. For example, H1 knockout mice have increased (but modest) non-REM sleep [20] ,as do animals injected with more selective second generation H1-antagonists, such as ketotifen [21]. At low doses, the first generation doxepin is highly selective for the H1-histamine receptor [22] and also increases sleep. Whether specific populations of H1-receptor expressing neurons are required for doxepin's sleep-promoting effects is unknown. However, direct injection of histamine into the wake-promoting basal forebrain in rat [23] or into the cat mesopontine tegmentum increases wakefulness, an effect that is strongly inhibited by H1 receptor antagonists [24]. Thus, histamine signalling in specific circuits is sufficient to drive wakefulness and may therefore be key targets of sleep-promoting H1 antagonists.

The histamine system also regulates wake-fulnesss in zebrafish. Morpholino knockdown of histamine decarboxylase reduces wakefulness [25], as do histamine receptor H1 antagonists, such as pyrilamine, chloropyramine, and loratadine [3**]. Given the molecular and anatomical conservation of the histamine system between fish and mammals, the zebrafish system is a good model for elucidating how histamine H1 antagonists regulate wakefulness [26].

Pitolisant (tiprolisant; BF2.649) is a histamine H3 inverse agonist/antagonist currently in Phase III clinical trials for the treatment of excessive daytime sleepiness and narcolepsy [27]. Histamine H3 receptors act as autoreceptors on histaminergic neurons, creating a negative feedback loop thought to reduce the synthesis and release of histamine [28, 29]. H3 autoreceptors on the wake-promoting TMN neurons (Figure 1A) are presumed to underlie H3 receptor antagonist's effects on waking, based on pharmacological and genetic evidence that show increased histaminergic tone and wakefulness [27–29]. However, histamine H3 receptors are expressed by most monoamine neurons and also can form heterocomplexes that regulate the function of many other neurotransmitter systems, including cholinergic, GABAergic, glutamatergic, and peptiderigic transmission [29]. Whether blockade of these other heteroreceptors contributes to the wake-promoting effects of H3 antagonists will require additional study. For both the histamine H1 receptor and H3 autoreceptors, locus-specific knockout and replacement studies are promising strategies to dissect these sites of action.

Adenosine Receptor Antagonists

A host of other sleep-regulatory substances, or somnogens, have been identified, including the cytokines interleukin-1 and tumor-necrosis factor alpha (TNF), prostaglandins, and adenosine [30, 31]. Although the proposed cascading interactions among these somnogens are complex, adenosine signalling is thought to be a major downstream effector for all of these signals [30–32].

Adenosine can signal through four adenosine receptors, of which the A1 and A2A receptors have been most strongly implicated in sleep regulation (reviewed in [32]). Likely produced in part by glia [33], endogenous adenosine levels in the cortex and especially the basal forebrain rise during wakefulness [34], where it is thought to inhibit wake-promoting cholinergic neurons via the inhibitory A1 receptor (Figure 1A)[32, 35]. Direct infusion of A1 receptor agonists into the BF increases sleep while both small molecule antagonists and locally delivered siRNA-mediated A1 receptor knockdown increase wakefulness [36, 37]. Similar pharmacological experiments have implicated other A1 receptor-expressing neurons, including the orexin neurons in the lateral hypothalamus [38] and the histaminergic neurons in the TMN as direct targets of adenosine's sleep signals [39]; however, the rise of adenosine levels during waking in the basal forebrain makes it a prime candidate for adenosine-mediated sleep homeostasis. Stimulatory A2A receptors are also thought to be important for adenosine's sleep effects in areas including the sleep-active VLPO and the basal ganglia, as perfusion of selective A2A receptor agonists into these areas promotes sleep and increases the activity of VLPO neurons [40–42].

Caffeine is an adenosine A1 and A2A receptor antagonist whose wake promoting effects are thought to be mediated by blocking the adenosine A2A receptor. A1 knockout mice are still aroused by caffeine whereas A2A knockouts are insensitive [43]. Does caffeine act on the wake- and sleep-promoting neurons proposed as loci for endogenous adenosine signalling, such as the basal forebrain? Remarkably, when adenosine A2A receptors in the shell of the nucleus accumbens (but not other areas) are deleted genetically in mice or by RNA interference in rats, the arousing effects of caffeine are blocked [44**]. This directly implicates classical motivational systems, including dopamine circuits (see below) in caffeine's wake-promoting effects and raises intriguing questions about how this system interacts with other sleep- and wake- promoting centers [45]. Because elimination of adenosine A2A receptors in the nucleus accumbens has no effect on baseline wakefulness, the extent to which adenosine signalling in this area regulates normal sleep/wake behavior is unclear. It is possible that small molecules like caffeine alter behaviour through

nonphysiological activation/inhibition of auxiliary arousal systems and not by direct modulation of core sleep circuitry *per se*.

Adenosine agonists increase sleep, while adenosine antagonists decrease sleep in other model species, including zebrafish [3**] and *Drosophila* [46]. Intriguingly, *Drosophila* mutants lacking the adenosine receptor have normal sleep and caffeine responses, and caffeine may act instead through inhibition of cAMP phosphodiesterase, which is also a strong caffeine binding target in mammals [46]. Future work will clarify in these systems where and how adenosine regulates evolutionarily conserved sleep processes, such as homeostasis.

Dopamine transporter antagonists

Modafinil and the related armodafinil are small molecules that promote wakefulness in many species and are used clinically in the treatment of excessive daytime sleepiness and narcolepsy. Although the only significant binding affinities demonstrated for modafinil are to the dopamine transporter (DAT) and, more weakly, the norepinephrine transporter (NET), a mechanism of action involving inhibition of DAT remains controversial, mainly because modafinil appears to lack the addiction potential of other DAT inhibitors, such as amphetamine, methylphenidate, and cocaine. Nevertheless, experimental evidence continues to support dopamine modulation as modafinil's mechanism of action. DAT knockout mice are unresponsive to modafinil [47], as are mutant mice lacking dopamine D1 and D2 receptor signalling [48], and some aspects of modafinil-induced arousal depend on the dopamine D4 receptor [49]. In vivo binding studies using positron emission tomography (PET) in monkeys [50] and humans [51], as well as direct dopamine measurements in monkey brain [52] are consistent with modafinil inhibition of DAT. Why modafinil lacks the potent addiction potential of other psychostimulants remains a mystery, but structure/binding studies on wild type and mutant DATs show that modafinil binding to the transporter is mechanistically distinct from cocaine [53], possibly altering functional outcomes.

Which dopamine circuits participate in mammalian sleep/wake regulation is unknown, but experiments in *Drosophila* demonstrate that a discrete dopamine circuit controls wakefulness independently from other behaviours, such as situational arousal. DAT mutant flies are short sleepers [54, 55], while D1 dopamine receptor loss-of-function mutants (DopR) are long sleepers [56]. The short-sleeping mutant, *insomniac*, in which the adaptor for the E3 ubiquitin ligase, Cullin-3, is disrupted, also has upregulated dopamine signalling [57, 58]. Dopamine also increases the activity of the wake-promoting l-LNvs, an effect dampened by light [59]. Remarkably, studies using genetics and neural activation/imaging [60**, 61**] to map the critical dopamine sleep circuit converged onto only two dopamine neurons that signal to the fan shaped body, a brain area implicated in fly sleep/wake regulation and isoflurane-induced anaesthesia (Figure 1B) [62, 14]. This exquisite neuronal specialization underscores the notion that drug-induced behavioural changes may require activity at only a fraction of all drug-affected neurons.

Zebrafish is another potential model system for studying the role of modafinil and dopamine in sleep/wake regulation. Modafinil increases wakefulness of larval zebrafish [63], and dopamine receptor D1–D4 agonists and antagonists alter sleep/wake behaviour, albeit in a complex manner; for example, D2 receptor agonists such as pergolide increased sleep, while D2-receptor antagonists like droperidol paradoxically increased both sleep and locomotor activity during waking [3**]. Future studies in zebrafish will need to map which neurons are activated by modafinil and more carefully address the structure-activity relationship of

dopamine receptor drugs to better understand how dopamine is involved in zebrafish sleep [4].

Melatonin Receptor Agonists

Melatonin is a hormone produced in a circadian (about 24-hour) rhythm by the pineal gland (Figure 1A) and signals predominantly through two G-protein coupled receptors, melatonin receptors MT1 and MT2. Because melatonin is produced at night in both nocturnal and diurnal animals, and pinealectomy fails to consistently alter sleep [64], the necessity of melatonin signalling in the direct regulation of sleep remains controversial [65, 66]. However, several melatonin-based therapeutics for insomnia have emerged, including extended-release melatonin (Circadin) and ramelteon (TAK-375; Rozerem), and is often used as a jet-lag aid.

Recent work with knockout mice and selective MT receptor agonists predominantly implicate the MT2 receptor in mediating melatonin's soporific effects. Mice lacking MT1 receptors have less REM sleep, while MT2 knockouts have a modest reduction only in non-REM sleep [67]. However, double knockouts are only slightly more awake than wild type animals over 24-hours [67]. Consistently, the MT2 selective agonist IIK7, induces NREM sleep in rat [68], as does the MT2 selective compound UCM765 in mouse [69*]. In addition, UCM765's sedative effect is abolished in MT2, but not MT1, receptor knockout mice. MT1 and MT2 receptors are widely expressed in many brain areas, including the circadian pacemaking suprachiasmatic nucleus (SCN) and other hypothalamic areas, the hippocampus, cerebellum, retina, prefrontal cortex, and others [70].

Which neuronal populations are critical for melatonin's sleep effects is not known, although infusion of the MT2-selective drug UCM765 into the sleep-active reticular thalamic nucleus (Figure 1A) increased GABAergic neuron burst firing and NREM sleep [69]. Conversely, direct action of melatonin on the circadian pacemaker in the SCN has been proposed by numerous studies to mediate the phase-shifting effects of melatonin (reviewed in [71]). Thus, distinct target circuits may underlie melatonin's hypnotic and phase-shifting properties, although more work is needed to disentangle these processes. Viral-mediated focal replacement of functional melatonin receptors in mutant backgrounds will be an attractive experimental paradigm for dissecting these complexities. Additionally, as melatonin is strongly hyponotic in zebrafish larvae [3**, 72–73], the zebrafish may be an excellent model for disentangling melatonin's endogenous roles in sleep.

Orexin/hypocretin receptor antagonists

Orexin/hypocretin is a wake-promoting neuropeptide produced by a small number of neurons in the hypothalamus that send projections to many wake-promoting brain areas (Figure 1A). Dysfunction of the orexin system in humans leads to a sleep disorder, narcolepsy, associated with excessive daytime sleepiness, unstable sleep/wake transitions, and cataplexy. Orexin also promotes wakefulness in zebrafish; overexpression of orexin dramatically increases waking [74], orexin neurons are maximally active during the wake phase [75], and loss of orexin neurons leads to increased sleep/wake transitions [76]. Although Drosophila do not have the orexin system, the neuropeptide PDF may fulfill a conserved role in promoting wakefulness [16**, 17**]. In mammals, orexin signalling is mediated by two G-protein coupled receptors, orexin receptor 1 and 2 (OX-1 and OX-2, also called HcrtR-1 and -2). Recently, several dual OX-1/OX-2 antagonists have been developed as possible hypnotics, including almorexant (ACT-078573, but no longer clinically pursued; [77]) and suvorexant (MK-4305; [78]). At the end of 2012, Merck announced that suvorexant was accepted for FDA review based on promising clinical data that suvorexant improved sleep in patients [79, 80].

Pharmacological and receptor mutant analyses indicate that antagonism at the OX-2 receptor is particularly critical for non-REM sleep effects of these drugs. OX-2 receptor knockout mice have stronger phenotypes than OX-1 mutants [81, 82] and renders mice insensitive to the sleep effects of almorexant [83]. Consistently, OX-2 selective antagonists induce stronger sleep effects than OX-1 antagonists in both mice [84] and rats [85], although blocking both receptors may be even more effective [82, 84]. Viral-mediated replacement of OX-2 receptors in the histaminergic TMN of OX-2 knockout mice increases wake consolidation but does not rescue sleep fragmentation, indicating these phenotypes are regulated by discrete brain areas [86*]. Consistent with the idea that these are experimentally separable, expression of orexin peptide in the zona increta (which connects to the wake-promoting LC), or in the pons of orexin knockouts selectively rescues cataplexy but not wake or sleep fragmentation [87, 88].

Optogenetic studies of mouse orexin neurons and the noradrenergic LC demonstrate the importance of this particular connection in modulating sleep to wake transitions. Orexin neurons do fire predominately during wakefulness but also at a low frequency during sleep [89]. Optogenetic stimulation of either orexin or LC neurons during sleep shortens the latency to wakefulness, although orexin neuron activation wakes animals more slowly than LC stimulation [90, 91]. These neurons likely work in the same circuit, because stimulating orexin neurons while optogenetically blocking activity of the LC prevents the increases in sleep-to-wake transitions [92**]. Meanwhile, stimulation of orexin neurons in histamine deficient mice can still increase sleep-to-wake transitions [93], demonstrating that different sleep/wake functions can be ascribed to discrete orexin receptor-expressing neurons. More generally, optogenetically inhibiting and activating candidate sleep sub-circuits during drug exposure may be a powerful way to pinpoint neuronal drug targets without altering long-term brain activity, as in traditional knockout studies.

Prospects

Many of the experiments reviewed here point to the existence of discrete neuronal substrates as critical targets for small molecules' sleep effects and suggest that sleep drugs do not require global modulation of brain activity to regulate sleep. In mice, viral-mediated focal knockouts and replacement strategies combined with optogenetics avoid problems of developmental compensation often seen in whole animal knockouts, allowing specific circuits to be experimentally manipulated in adults. Expanding these local knockout strategies to study additional small molecules presents both an opportunity and a challenge, as success requires choosing the correct few neurons from the whole brain to manipulate. Targeting previously described sleep-wake regulatory neurons serves as a good starting point, but small molecules (e.g. benzodiazepines, caffeine, and modafinil) are implicating novel and unexpected circuits in sleep-and wake-promoting drug activity. Furthermore, potential redundancy among multiple sleep circuits poses an obstacle for a locus-by-locus approach.

Insights from non-mammalian model systems offer some clues as to the functional and neuroanatomical properties of the neurons targeted by sleep drugs. In *Drosophila*, identifying the neurons responsible for drugs' arousing or sedating actions provides a topology for how these circuits interact with other internal and external behavioural regulatory systems, such as food, light, motivational states, homeostatic processes, circadian rhythms, and memory formation. For example, linking the hypnotic effects of GABA directly onto the wake-promoting PDF cells in *Drosophila* [16**, 17**] highlights the intimate association between sleep and circadian rhythms and draws parallels to the mammalian circadian pacemaker in the SCN. Similarly, dopamine signalling networks,

which play a fundamental in *Drosophila* sleep [60, 61], should also receive more attention as direct sleep/wake regulators in mammals [45].

Zebrafish larvae are also well-suited for small molecule sleep research. Compounds can be directly delivered in the water in 96-well plates and reach the brain, as these larvae lack the blood-brain barrier at this time [4]., which facilitates screens for novel sleep/wake drugs. For example, one screen of nearly 6000 small molecules identified several hundred structures that reliably alter larval fish locomotor and sleep behavior [3**]. These small molecules included many of the well-established sleep drug classes discussed in this review, such as modulators of the GABA, dopamine, histamine, noradrenaline, adenosine, and melatonin systems, as well as numerous modulators of NfkB, which is a likely integrator of cytokine, prostaglandin, and adenosine signals [30, 31]. The screen also identified the ether-a-go-go gene related potassium channel (ERG) and L-type calcium channel inhibitors as novel regulators of sleep and wake [3**]. The challenge now is to link these myriad small molecules to specific molecular and neuronal substrates. Drug-activated neurons can be mapped using immediate early gene expression [94] or by direct calcium imaging of neuronal dynamics in the whole brain [95**]. Once key drug-altered neurons are identified, they can be ablated by chemical genetics with nitroreductase [76] or by laser [96] and optogeneticallly modulated [97] to test their necessity in both drug-induced and normal sleep. Because of the considerable homology between zebrafish and mammalian brain structures, such a sleep-drug activation map in fish could have a direct correspondence to key sleep circuitry in mammals. In addition, the low cost and high-thoughput afforded by the simpler zebrafish and *Drosophila* models facilitate both drug discovery screens as well as the functional characterization of novel compounds [4].

In conclusion, a greater understanding of the molecular and neuronal substrates for major sleep and wake drugs not only will advance our knowledge of how sleep is regulated, it could also aid in the development of more target-selective sleep therapeutics. While this search continues using the techniques of modern mouse genetics, insights from non-mammalian systems, such as flies and fish, will also facilitate targeted approaches in mammals by pinpointing critical features likely to be shared by sleep circuits in all species.

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GABA-A receptor gene, *Resistant to dieldrin* (*Rdl*) specifically in PDF neurons reduced total sleep. The small and large ventral lateral neurons (LNvs), circadian clock cells that express PDF form a wake-promoting circuit, as mutants lacking PDF the receptor, or the PDF neurons sleep more. Parisky et al further showed that specifically manipulating the excitiability of these neurons alters sleep. Together, these data suggest a model similar to the mammalian “flip-flop” switch, with the wake-promoting LNvs inhibited by (as yet unidentified) sleep-promoting GABAergic inputs.

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Highlights

1. The mechanisms and sites of action for sleep-altering drugs are poorly understood
2. Conditional mouse mutants and optogenetics are identifying critical sleep circuits
3. Sleep drugs may act on critical sets of neurons, instead of globally
4. Small molecules affect Zebrafish and *Drosophila* sleep in conserved ways

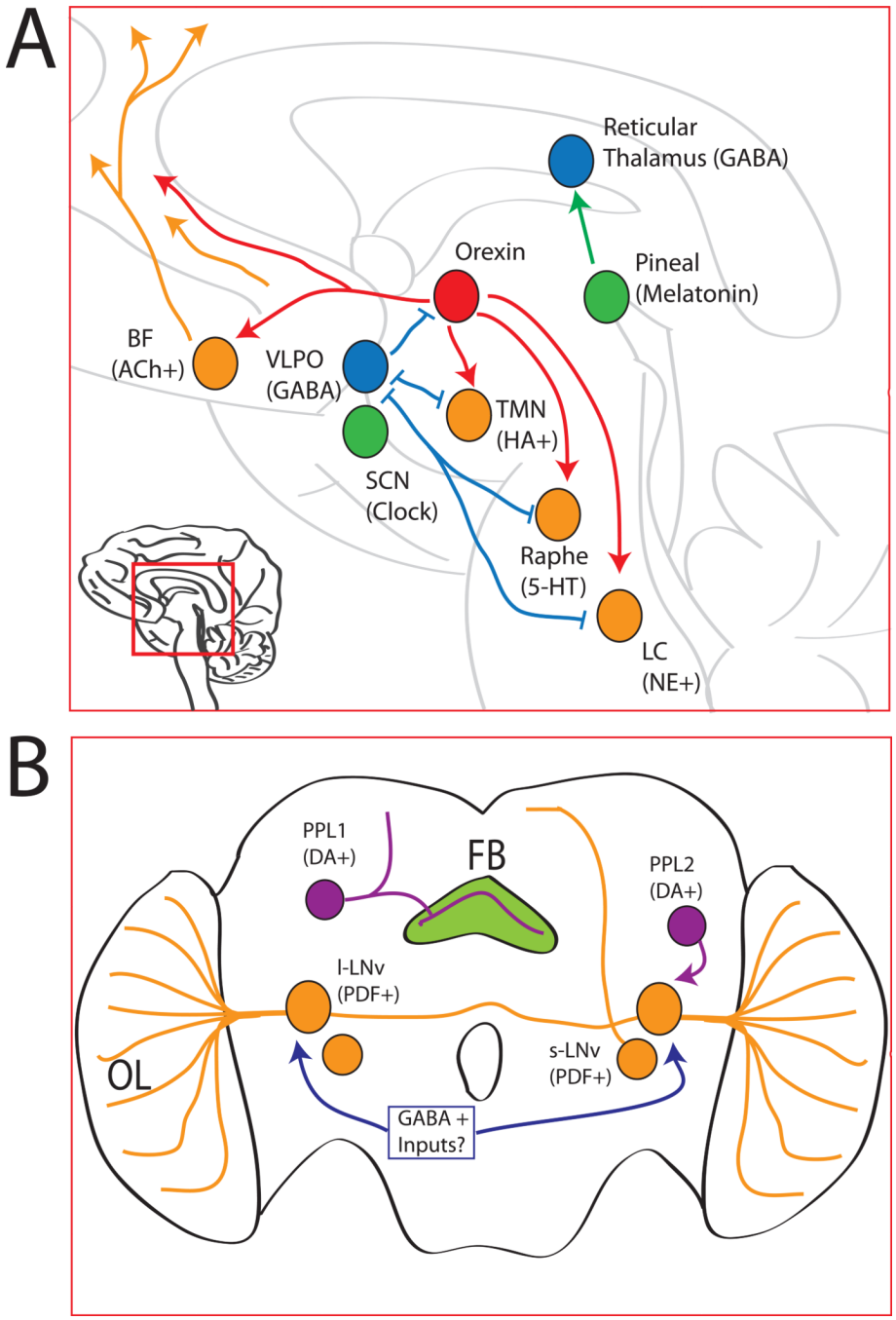

Figure 1. Major Sleep-Wake Pathways in Mammals and Flies

A) The major mammalian sleep/wake regulatory pathways discussed in this review are shown. The ascending arousal system (ORANGE) is made up of many wake-promoting circuits, including the cholinergic basal forebrain (BF), the histaminergic (HA) tuberomammillary nucleus (TMN), the seroternergic (5-HT) dorsal raphe, and the noradrenaline (NE) producing locus coeruleus (LC). These areas send arousing projections (ORANGE LINES) to the thalamus and neocortex. The orexin neurons (RED) send excitatory projections to the ascending arousal network. The GABAergic neurons of the ventrolateral preoptic nucleus (VLPO; in BLUE), which makes inhibitory connections with both the orexin and ascending arousal systems. The TMN, raphe, and LC make mutual

inhibitory connections with the VLPO to form a 'flip-flop' circuit. The pineal gland (GREEN) is the major source of melatonin, which can signal to the suprachiasmatic nucleus (SCN), which is the master regulator of circadian rhythms in mammals, and the GABA-positive reticular thalamus (BLUE), which may contribute to melatonin's hypnotic effects. See text for details.

B) The major *Drosophila* circuits discussed in this review are shown on this schematic fly brain. Although not shown, all structures are bilaterally symmetric. In ORANGE are the major clock neurons, the large and small ventral lateral neurons (l-LNv and s-LNv). The wake promoting l-LNvs connect to the contralateral optic lobe (OL). Inhibitory GABA inputs (BLUE) are postulated for the l- LNvs. A single dopamine (DA) neuron (PURPLE) in the protocerebral posteriolateral 1 cluster (PPL1) signals to the fan shaped body (FB; GREEN) to increase *Drosophila* wakefulness. The wakepromoting l-LNvs also receive dopamine inputs, including from, but not limited to, the PPL2 cluster. See text for details.

Table 1

Molecular and neuronal targets of small molecule sleep regulators.

			Sleep Effects in:		
Name/Class	**Molecular Target/Activity**	**Putative Neuronal Sleep Target(s)**	**Mammals**	**Zebrash**	**Drosophila**
Z-drugs Benzodiazepines	GABA-A agonist (α1 subunit) GABA-A agonist (α1,2,3,5)	Ascending Arousal System (TMN, LC, dorsal raphe) Amygdala?	increased sleep	increased sleep	increased sleep
Doxepin	Histamine H1 receptor antagonist	Basal Forebrain, Cortex	increased sleep	increased sleep	?
Pitolisant	Histamine H3 receptor antagonist	TMN	increased wake	?	?
Adenosine	Adenosine Receptor A1, A2A Agonist	Basal Forebrain, Cortex	increased sleep	increased sleep	increased sleep
Caffeine	Adenosine Receptor A2A Antagonist	Shell of Nucleus Accumbens	increased wake	increased wake	increased wake
Modafinil	dopamine transporter (DAT)Inhibitor	?	increased wake	increased wake	increased wake
Melatonin	Melatonin receptor MT1/MT2 agonist	SCN, Reticular Thalamus	increased sleep	increased sleep	N/A
Almorexant Suvorexant	Orexin receptor OX1/OX2 antagonist	Ascending Arousal System (TMN, LC, raphe)	increased sleep	(orexin/hcrt increases wake)	N/A

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