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Sites of action of sleep and wake drugs: insights from model organisms

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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.

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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-promoting and wake-promoting drugs, much remains unknown about the basic mechanisms and sites of action by which these drugs work [1].

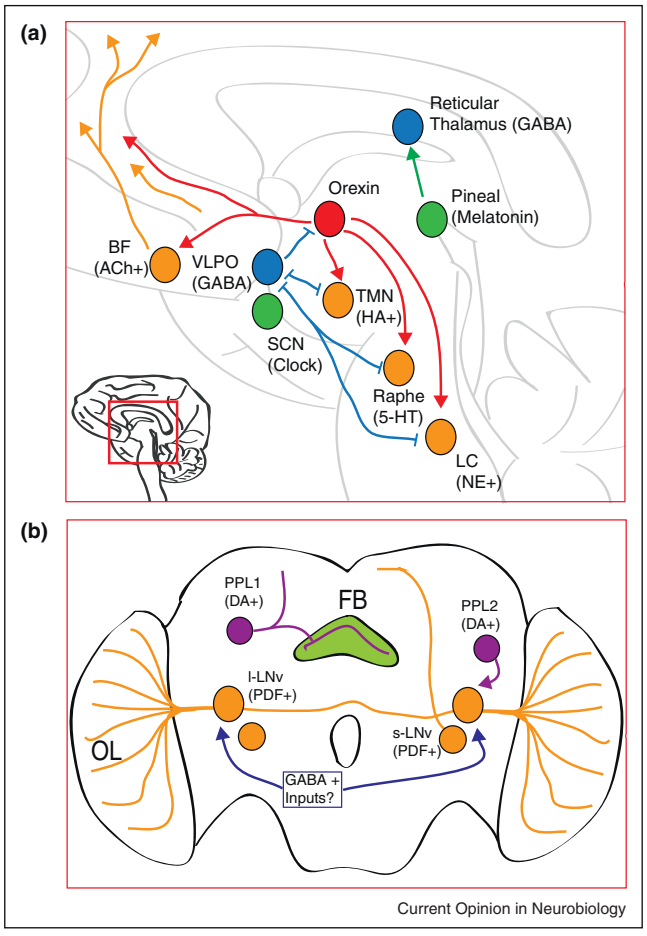
There are many reasons for the gap in understanding. Sleep and arousal are highly complex brain states, involving many different neural circuits and neurotransmitter systems. In some cases, these sleep-promoting drugs have significant affinity for multiple protein targets, and each of which may have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

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) make 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 wake-promoting l-LNvs also receive dopamine inputs, including from, but not limited to, the PPL2 cluster. See text for details.

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 centres 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 (Figure 1a) 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*