Pharmacosynthetic Deconstruction of Sleep-Wake Circuits in the Brain

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Abstract

Over the past decade, basic sleep research investigating the circuitry controlling sleep and wakefulness has been boosted by pharmacosynthetic approaches, including chemogenetic techniques using designed receptors exclusively activated by designer drugs (DREADD). DREADD offers a series of tools that selectively control neuronal activity as a way to probe causal relationship between

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neuronal sub-populations and the regulation of the sleep-wake cycle. Following the path opened by optogenetics, DREADD tools applied to discrete neuronal sub-populations in numerous brain areas quickly made their contribution to the discovery and the expansion of our understanding of critical brain structures involved in a wide variety of behaviors and in the control of vigilance state architecture.

Keywords

Arousal · Chemogenetic · Designer Receptor Exclusively Activated by Designer Drugs (DREADD) · Non-REM sleep · Paradoxical sleep · Pharmacogenetic · REM sleep · Slow-wave sleep · Wakefulness

1 Introduction

The development of new hypnotic and wake-promoting medications relies on a solid understanding of the neurochemical mechanisms and brain circuits regulating sleep and wakefulness to improve specificity of action with enhanced safety and efficiency. Over the past decade, basic sleep research investigating the circuitry controlling sleep and wakefulness has been boosted by pharmacosynthetic approaches, some of which can be perceived as an ideal pharmacotherapeutic strategy involving G-protein-coupled receptor (GPCR) signaling. Indeed GPCRs represent the largest class of neuronal signal-transducing molecules capable of modulating neuronal firing (see for review Farrell and Roth 2013) and have proven to be the most highly favorable class of drug targets in modern pharmacology (Drews 2000; Wise et al. 2004). In this context, the development of *d*esigned *r*eceptors *e*xclusively *a*ctivated by *d*esigner *d*rugs (DREADDs) has already proven its relevance to molecular therapy implicating GPCR-linked pathways in the modulation of the circadian rhythm and sleep-wake cycle and in many behaviors and disorders including eating- and addictive-related behaviors or autonomic dysfunctions (see for review Urban and Roth 2015).

Similar to optogenetic approaches (see Adamantidis and Lüthi 2018), the primary goal of DREADD technique applied to systems neuroscience is to offer a series of tools that selectively control neuronal activity as a way to probe causal relationship between neuronal subpopulations and behavior. Its basic principle consists in introducing exogenous genes into neurons that enable them to respond to specific exogenous chemical ligands that have no interfering pharmacological effect. These tools provide a powerful combination of genetic specificity and spatial precision for a potential multiplexed control (multiple chemogenetic actuators controlling simultaneously or sequentially different neuronal pathways) that are critical for a systems-level understanding of brain functions. However, in contrast with optogenetics, DREADD technique lacks a temporal resolution to control the activation or silencing of neurons within milliseconds. On the other hand, it further mimics longer-term modulation of neuronal signaling associated with pharmacotherapeutic strategies targeting GPCRs (see for review Urban and Roth 2015) and could be better suited for large brain structures instead of volume-limited control through optic fibers. The

expansion of pharmacosynthetic approaches not only offers multiple ways to control neuronal excitability but also allows to study and control gene transcription and translation, protein-protein interactions, enzymatic function, and protein stability (see for review Sternson and Roth 2014).

This chapter first draws up the principles of the DREADD technology that has been the most widely used in sleep research, followed by a discussion on the potential limitations and concerns that were recently raised in the literature. This section intends to identify and clarify the risks associated with DREADDs in order to control and support future experiments and bring a better perspective at the reading of the findings emerging from DREADDs in sleep research. Next we will review the experiments deconstructing arousal- and sleep-promoting circuits and their resulting discoveries and discuss their significance in the face of past knowledge and future directions.

2 Description of Pharmacosynthetic Tools

Two techniques emerged over the past decade to remotely control neuronal activity in vivo: optogenetics based on engineered opsins (see Adamantidis and Lüthi 2018) and pharmacosynthetic approaches including DREADD-based chemogenetics utilizing engineered GPCRs and *p*harmacologically *s*elective *a*ctuator *m*olecules (PSAM) utilizing chimeric *l*igand-*g*ated *i*on *c*hannels (LGIC) that both selectively interact with synthetic small molecules (see for review Farrell and Roth 2013; Sternson and Roth 2014). In basic sleep research using rodent models, the DREADD technology referred to as the third generation of muscarinic GPCR-based chemogenetics (Armbruster et al. 2007) has been the most broadly adopted tool.

2.1 Principles of the DREADD Technology

The DREADDs were first generated from the human M3 muscarinic receptors subjected to random mutagenesis in the yeast and screened from absence of drug-dependent growth assays in the presence of clozapine N-oxide (CNO). Resulting surviving mutants (hM3DGq) evolve receptors that are activated only by CNO and lack activation by their native ligand, acetylcholine (ACh) (Dong et al. 2010). CNO is an inactive metabolite of the atypical antipsychotic clozapine. It was chosen for its ability to penetrate the central nervous system and for its favorable pharmacokinetics and inert pharmacology (Bender et al. 1994; Armbruster et al. 2007). However, few reports raised concerns that CNO could be interconverted to clozapine in various species (Loffler et al. 2012; Gomez et al. 2017). While a small fraction of interconverted CNO is established in humans, primates, and guinea pigs, the occurrence of this process in rodents is still debated and is further addressed in the next section. Still in vitro, CNO activation of the hM3DGq mutant in transfected neurons triggers Gq-coupled signaling leading to membrane depolarization through

phospholipase C/PIP2-mediated inhibition of voltage-activated KCNQ potassium channels (Armbruster et al. 2007; Alexander et al. 2009).

Using a similar design scheme, a second mutated human M4 receptor coupled to Gi proteins (hM4DGi) was generated leading to neuronal hyperpolarization mediated by G-protein-coupled inwardly rectifying potassium channels (GIRKs) upon CNO application (Armbruster et al. 2007; Zhu et al. 2014). In vivo validation of DREADD-driven neuronal activation or inhibition has been reported in numerous studies conjointly with major behavioral consequences (see for review Sternson and Roth 2014). However so far, only few groups combined CNO administration and direct in vivo unit recordings to examine and directly control CNO-driven DREADD effects on the spontaneous neuronal activity and firing discharge. Most of these studies confirmed modifications of neuronal activities by ex vivo electrophysiological recordings in brain slices or by indirect methods involving the labeling of the cFos immediate early gene, as a marker of neuronal activation.

A third DREADD construct was generated from a chimeric muscarinic-adrenergic receptor that selectively activates Gαs signaling pathways (rM3/β1Gs) promoting cAMP production through the activation of adenylate cyclase (Guettier et al. 2009; Farrell et al. 2013). Its first use was applied to pancreatic β-cells in vitro and in vivo to deconstruct insulin secretion signaling pathway (Guettier et al. 2009). Unlike hM3DGq or hM4DGi receptors, a small degree of constitutive activity from transfected cells has been reported with rM3/β1Gs leading to a modest basal phenotype (Guettier et al. 2009). Interestingly, the specific activation of the different G-coupled pathways using DREADD approaches was interrogated to deconstruct the intracellular control of circadian pacemaking activity of suprachiasmatic nucleus (SCN) neurons in vitro (Brancaccio et al. 2013). This study showed that, although CNO-mediated induction of Gs significantly increases calcium/cAMP-responsive elements in a larger degree than Gq, the activation of Gq-dependent pathway in a subset of SCN neurons, but not Gs or Gi, selectively reprograms the circadian oscillations of intracellular calcium and transcriptional/posttranslation feedback loop rhythms in the entire SCN (Brancaccio et al. 2013). This reorganization requires a VIPergic signaling revealing a Gq-intracellular calcium-VIP leitmotif that determines the intrinsic network encoding of SCN circadian time (Brancaccio et al. 2013). In addition to a broad remote control of neuronal activity, this work illustrates a major potential of DREADDs to interrogate the relationship between G-coupled pathways, cytosolic signaling, and the transcriptional feedback loops with neuronal activity.

The DREADD genes are expressed via transgenic or viral approaches for conditional gene targeting. The most commonly used method to obtain high-expression levels of DREADD consists in Cre-dependent viruses carrying the DREADD transgene under the control of strong ubiquitous promoters (such as EF1α, hSyn) injected in specific Cre-driver lines. The vast library of Cre-driver knock-in and bacterial artificial chromosomes (BAC) transgenic mice available today offers thousands of targeting possibilities. Still confirmation of the specificity of the Cre expression in the desired genetically defined cell population by the absence of ectopic expression at least in the region of interest when using viral vector approach is required. The

activation of GPCR signaling pathways using DREADDs has revealed remarkably specific behavioral and physiological influences for a variety of neuronal, but also non-neuronal, cell types that are often intermingled with populations having different or even opposite functions (see for review Sternson and Roth 2014; Roth 2016). Sleep studies using DREADDs are further detailed in the next sections (Sect. 3) and listed in Table 1. The main advantages of this technology are as follows: (1) it can be minimally invasive as CNO can be administered orally in drinking water; (2) CNO kinetics involve a prolonged modulation of neuronal activity (minutes to hours) with physiological relevance to canonical and conserved GPCR signaling pathways; (3) there is no specialized equipment required as all chemical actuators are commercially available; (4) it allows a large-scale control of a diffuse neuronal ensemble, which is challenging to illuminate with optogenetics, while it still allows subdomain specific targeting with local injections; and finally (5) it is a relatively inert intervention upon the CNO doses used. The main disadvantage of the DREADD system is the lack of precise temporal control in comparison with what is achieved with optogenetics. Compounds with enhanced pharmacokinetic properties and light-activated photocaged compounds allowing for more precise control over time of the DREADD receptor activation are still under development (see for review Sternson and Roth 2014). Other disadvantages related to CNO and inherent properties of GPCRs are further discussed in the following sections (Sects. 2.2 and 2.3).

2.2 What to Keep in Mind Before DREADD Use?

For a comprehensive understanding of the DREADD technology and a more effective use, inherent properties of GPCRs should be kept in mind. Many GPCRs show a considerable amount of basal, ligand-independent activity implying that a GPCR can activate its G protein in the absence of an agonist (see for review Kobilka and Deupi 2007). Therefore a major concern for chemogenetic approaches is the possibility that high levels of expression of an engineered protein might have effects in the absence of chemical activation (Conklin et al. 2008). This constitutive activity has been questioned and examined for DREADD receptors. To date, none of the studies using hM3DGq or hM4DGi have reported a basal phenotype implying no detectable constitutive activity. Lifelong and considerably high levels of expression that were attained using either a genetically encoded tetracycline-sensitive induction system or viral transduction of hM3DGq or hM4DGi were not reported to have any abnormality from basal electrophysiological, behavioral, or anatomical characterizations (Alexander et al. 2009; Zhu et al. 2014; see for review Roth 2016). However, the lack of report does not imply the absence of basal activity. A relatively low constitutive activity was found with the rM3/β1Gs construct looking at cAMP and inositol phosphate basal levels from transfected cells, which were not apparent with hM3DGq (Guettier et al. 2009). However, no detectable electrophysiological, behavioral, or anatomical phenotype was reported in a rM3/β1Gs transgenic mouse (Farrell et al. 2013). To counteract this issue if any basal activity is observed, cautions on titrating

Table 1 Chemogenetic control of brain regions/neurotransmitter systems and effects on sleep-wake behavior

Authors	Brain area	Animal model[a]	DREADD-containing vector[b]	CNO dose	Effects on vigilance states[c]
Medulla					
Chen et al. (2017) Experimental neurology	RVM	Sprague-Dawley rats	AAV_{10}-Cre + AAV_{10}-hSyn-DIO-hM3Dq-mCherry	0.2 mg/kg	↑ Wake; ↓ SWS; ↓ REM
	pSOM	Sprague-Dawley rats	AAV_{10}-Cre + AAV_{10}-hSyn-DIO-hM3Dq-mCherry	0.2 mg/kg	↔ Wake; ↔ SWS; ↓ REM
	pSOM (GABA)	*Vgat-IRES-Cre* mice	AAV_{10}-hSyn-DIO-hM3Dq-mCherry	0.3 mg/kg	↔ Wake; ↔ SWS; ↓ REM
Anaclet et al. (2014) Nat Neurosci	PZ (GABA)	*Vgat-IRES-Cre* (129/B6/FVB) mice	AAV_{10}-hSyn-DIO-hM3Dq-mCherry	0.3 mg/kg	↓ Wake; ↑ SWS; ↓ REM
			AAV_{10}-hSyn-DIO-hM4Di-mCherry	0.3 mg/kg	↑ Wake; ↓ SWS; ↓ REM
Pons					
Hayashi et al. (2015) Science	Medial Atoh1-E10.5 neurons	*Atoh1-CreER^{T2}*; *CAG-LSL-tTA* (B6) mice (tamoxifen @E10.5)	AAV_5-TRE-hM3Dq-mCherry	1.5 mg/kg	↔ Wake; ↑ SWS; ↓ REM
	Lateral Atoh1-E10.5 neurons	*Atoh1-CreER^{T2}*; *CAG-LSL-tTA* (B6) mice (tamoxifen @E10.5)	AAV_5-TRE-hM3Dq-mCherry	1.5 mg/kg	↑ Wake; ↓ SWS; ↓ REM
	Medial Atoh1-E10.5 neurons (glutamate)	*Atoh1-CreER^{T2}*; *Camk2a-LSL-tTA* (B6) mice (tamoxifen @E10.5)	AAV_5-TRE-hM3Dq-mCherry	1.5 mg/kg	↔ Wake; ↔ SWS; ↓ REM
		Atoh1-CreER^{T2}; *Vglut2-LSL-tTA* (B6) mice (tamoxifen @E10.5)	AAV_5-TRE-hM3Dq-mCherry	1.5 mg/kg	↔ Wake; ↔ SWS; ↓ REM
	Lateral Atoh1-E10.5 neurons (glutamate)	*Atoh1-CreER^{T2}*; *Camk2a-LSL-tTA* (B6) mice (tamoxifen @E10.5)	AAV_5-TRE-hM3Dq-mCherry	1.5 mg/kg	↑ Wake; ↓ SWS; ↓ REM

	dDpMe (GABA)	*Vgat-Cre* KI (B6) mice	AAV_8- EF1α-DIO-hM3Dq-mCherry	1.5 mg/kg	↔ Wake; ↑ SWS; ↓ REM
		Vgat-Cre Tg (B6) mice	AAV_8- EF1α-DIO-hM3Dq-mCherry	1.5 mg/kg	↔ Wake; ↑ SWS; ↓ REM
		Vgat-Cre KI (B6) mice	AAV_8- EF1α-DIO-hM4Di-mCherry	1.5 mg/kg	↓ Wake; ↔ SWS; ↑ REM
		Vgat-Cre Tg (B6) mice	AAV_8- EF1α-DIO-hM4Di-mCherry	1.5 mg/kg	↔ Wake; ↔ SWS; ↑ REM
Qiu et al. (2016a) Curr Biol	PB	Sprague-Dawley rats	AAV_{10}-EF1α-hM3dq-mCherry	0.2 mg/kg	↑ Wake (11 h long); ↓ SWS; ↓ REM
	PB projecting to LPO	Sprague-Dawley rats	$AAV_{2/6}$-CAG-Cre (in LPO) and AAV_{10}-hSyn-DIO-hM3Dq-mCherry	0.2 mg/kg	↑ Wake; ↓ SWS; ↓ REM
	PB projecting to LH	Sprague-Dawley rats	$AAV_{2/6}$-CAG-Cre (in LH) and AAV_{10}-hSyn-DIO-hM3Dq-mCherry	0.2 mg/kg	↑ Wake; ↓ SWS; ↓ REM
	PB projecting to thalamus	Sprague-Dawley rats	$AAV_{2/6}$-CAG-Cre (in thalamus) and AAV_{10}-hSyn-DIO-hM3Dq-mCherry	0.2 mg/kg	No effect
Kaur et al. (2017) Neuron	PBel (CGRP)	*CGRP-CreER* (B6) mice (tamoxifen treatment prior to AAV injections)	$AAV_{2/10}$-hSyn-DIO-hM3Dq-mCherry	0.1–0.3 mg/kg	↑ Wake; ↓ SWS; ↓ REM
Kroeger et al. (2017) J Neurosci	PPT (acetylcholine)	*ChAT-IRES-Cre* (B6) mice	AAV_{10}-EF1α-DIO-hM3Dq-mCherry	0.3 mg/kg	↔ Wake; ↓ deep SWS; ↑ light SWS; ↔ REM
	PPT (glutamate)	*Vglut2-IRES-Cre* (B6) mice	AAV_8-EF1α-DIO-hM3Dq-mCherry (note different serotypes)	0.3 mg/kg	↑ Wake; ↓ SWS; ↓ REM
	PPT (GABA)	*Vgat-IRES-Cre* (B6) mice	AAV_8-EF1α-DIO-hM3Dq-mCherry	0.3 mg/kg	↑ REM

(continued)

Table 1 (continued)

Authors	Brain area	Animal model[a]	DREADD-containing vector[b]	CNO dose	Effects on vigilance states[c]
	PPT (glutamate)	*Vglut2-IRES-Cre* (B6) mice	AAV_8-EF1α-DIO-hM4Di-mCherry	1 mg/kg	↓ Wake; ↑ SWS; ↔ REM
Vazey and Aston-Jones (2014) Proc Natl Acad Sci U S A	LC (noradrenaline)	Long-Evans rats	$AAV_{2/9}$-PRSx8-HA-hM3Dq (PRSx8: adrenergic neurons promoter)	0.1, 1, 10 mg/kg/intracerebral 5 μM (30–60 nL)	↓ Emergence from general anesthesia; ↑ cortical arousal; ↓ Delta power; ↑ theta power during anesthesia
Hasegawa et al. (2014) J Clin Invest	DR (5HT) ↔ LH (orexin)	*Orexin/ataxin-3* (B6) mice (postnatal degeneration)	AAV_2-Pet1-HA-hM3Dq (Pet1: serotonergic-specific transcription factor)	5 mg/kg	↓ Cataplexy; ↑ REM latency; ↓ wake episode duration
	LC (noradrenaline) ↔ LH (orexin)	*Orexin/ataxin-3* (B6) mice	AAV_2-PRSx8-HA-hM3Dq (PRSx8: adrenergic neurons promoter)	5 mg/kg	↔ Cataplexy; ↑ wake episode duration; ↓ wake episode number
Midbrain					
Sun et al. (2017) Sleep Biol Rhythms	VTA	WT (129) mice	AAV_{10}-hSyn-hM3Dq-mCherry	1 mg/kg	↑ Wake; ↓ SWS; ↓ REM
			AAV_{10}-hSyn-hM4Di-mCherry	1 mg/kg	No effect
Eban-Rothschild et al. (2016) Nat Neurosci	VTA (dopamine)	*Th-IRES-Cre* KI (B6) mice	AAV_5-EF1α-DIO-hM4Di-mCherry	1 mg/kg	↓ Wake; ↑ SWS; ↑ REM
Oishi et al. (2017) Brain Struc Funct	VTA (dopamine)	*Dat-Cre* KI (B6) mice	AAV_{10}-hSyn-hM3Dq-mCherry	0.3 mg/kg	↑ Wake
				0.3 mg/kg + D_1R antago (SCH23390)	↑ Wake
				0.3 mg/kg + D_2R/D_3R	↔ Wake

				antago (racloprine)	
			AAV_{10}-hSyn-hM4Di-mCherry	0.3, 1 mg/kg	No effect
	SNc (dopamine)	*Dat-Cre* KI (B6) mice	AAV_{10}-hSyn-hM3Dq-mCherry	0.3 mg/kg	No effect
Hypothalamus					
Pedersen et al. (2017) Nat Commun	SUM (glutamate)	*Vglut2-IRES-Cre* (B6) mice	$AAV_{2/10}$-hSyn-DIO-hM3Dq-mCherry	0.3 mg/kg	↑ Wake (large)
		Vglut2$^{lox/lox}$ (B6) mice	AAV_{10}-hSyn-Cre-GFP and $AAV_{2/10}$-hSyn-DIO-hM3Dq-mCherry	0.3 mg/kg	↑ Wake (midl)
		Vglut2-IRES-Cre (B6) mice	$AAV_{2/10}$-hSyn-DIO-hM4Di-mCherry	0.3 mg/kg	↓ Wake; ↑ SWS; ↓ REM
	SUM (GABA)	*Vgat-IRES-Cre* (B6) mice	$AAV_{2/10}$-hSyn-DIO-hM3Dq-mCherry	0.3 mg/kg	↑ Wake
	SUM (glutamate/ NOS1)	*NOS1-IRES-Cre* (B6) mice	$AAV_{2/10}$-hSyn-DIO-hM3Dq-mCherry	0.3 mg/kg	↑ Wake
		NOS1-IRES-Cre (B6) mice	$AAV_{2/10}$-hSyn-DIO-hM4Di-mCherry	0.3 mg/kg	No effect on sleep/ wake; ↓ REM theta power
Venner et al. (2016) Curr Biol	LH (GABA)	*Vgat-IRES-Cre* (129/B6/FVB) mice	AAV_{10}-hSyn-DIO-hM3Dq-mCherry	0.3 mg/kg	↑ Wake; ↓ SWS; ↓ REM
			AAV_{10}-hSyn-DIO-hM4Di-mCherry	0.3 mg/kg	↓ Wake; ↑ SWS; ↔ REM
Sasaki et al. (2011) PLoS One	LH (orexin)	*Orexin-cre* (B6) mice	AAV_2-EF1α-DIO-HA-hM3Dq	5 mg/kg	↑ Wake; ↓ SWS; ↓ REM
			AAV_2-EF1α-DIO-HA-hM4Di	5 mg/kg	↓ Wake; ↑ SWS; ↓ REM
Vetrivelan et al. (2016) Neuroscience	LH (MCH)	*MCH-cre* mice	AAV_8-hSyn-DIO-hM3Dq-mCherry	0.3 mg/kg	↑ REM

(continued)

Table 1 (continued)

Authors	Brain area	Animal model[a]	DREADD-containing vector[b]	CNO dose	Effects on vigilance states[c]
Varin et al. (2018) Sleep	LH (MCH)	*MCH-cre* (B6) mice	AAV_{10}-hSyn-DIO-mCherry	0.5–10 mg/kg	↑ SWS; ↓ REM
			AAV_{10}-hSyn-DIO-hM3Dq-mCherry	0.5–1 mg/kg	↓ SWS duration; ↑ SWS depth; ↑ REM
			AAV_{10}-hSyn-DIO-hM4Di-mCherry	1–5 mg/kg	↑ SWS duration; ↓ SWS depth; ↔ REM
Yu et al. (2015) Neuron	TMN (histamine)	*HDC-cre* (129/B6) mice	$AAV_{1/2}$-hSyn-DIO-hM3Dq-mCherry	5 mg/kg	↑ Motor activity
Kodani et al. (2017) J Neurosci	BNST (GABA)	*GAD67-Cre* (B6) mice	AAV_{10}-EF1α-DIO-hM3Dq-mCherry	5 mg/kg	↑ Wake; ↓ SWS; ↓ REM
Saito et al. (2013) Front Neural Circuits	Preoptic area (GABA)	*Gad67-Cre* (B6) mice	AAV_2-EF1α-DIO-HAhM3Dq	5 mg/kg	↓ Wake; ↑ SWS; ↔ REM
Zhang et al. (2015) Nat Neurosci	Preoptic area – LPO or MnPO	WT (B6) mice	$AAV_{1/2}$-ITR-P_{cFos}-tTA-WPRE-pA-ITR and $AAV_{1/2}$-ITR-$P_{TRE\text{-}tight}$-hM3Dq-mCherry (doxycycline removal before dexmedetomidine anesthesia or sleep-deprivation-induced hypersomnia)	5 mg/kg	↓ Locomotor activity; ↑ SWS
Basal forebrain					
Anaclet et al. (2015) Nat Commun	VP, SI, MCPO, HBD (acetylcholine)	*ChAT-IRES-Cre* mice	$AAV_{2/10}$-hSyn-DIO-hM3Dq-mCherry	0.3 mg/kg	No effect on sleep/wake ↓ SWS EEG power
	VP, SI, MCPO, HBD (glutamate)	*Vglut2-IRES-Cre* mice	$AAV_{2/10}$-hSyn-DIO-hM3Dq-mCherry	0.3 mg/kg	No effect on sleep/wake ↓ SWS EEG power
	VP, SI, MCPO, HBD (GABA)	*Vgat-IRES-Cre* mice	$AAV_{2/10}$-hSyn-DIO-hM3Dq-mCherry	0.3 mg/kg	↑ Wake; ↓ SWS; ↓ REM ↑ Wake fast rhythms
			$AAV_{2/10}$-hSyn-DIO-hM4Di-mCherry	0.3 mg/kg	↓ Wake; ↑ SWS

	Thalamus (glutamate)	*Vglut2-IRES-Cre* mice	$AAV_{2/10}$-hSyn-DIO-hM3Dq-mCherry	0.3 mg/kg	No effect on sleep/wake ↑ Fast rhythms
Chen et al. (2016) Neuropsychopharmacology	MCPO, HDB, SI (acetylcholine)	*ChAT-IRES-Cre* (B6) mice	AAV-hSyn-DIO-hM3Dq-mCherry	0.1, 0.3, 1 mg/kg	↑ Wake; ↓ SWS; ↓ REM ↓ SWS delta power
			AAV-hSyn-DIO-hM4Di-mCherry	1 mg/kg	↓ Wake; ↑ SWS; ↓ REM ↑ SWS delta power
Subthalamus					
Liu et al. (2017) Nature	ZI (Lhx6)	*Lhx6-Cre* (B6) mice	AAV_9-EF1α-DIO-hM3Dq-mCherry	0.5 mg/kg	↓ Wake; ↑ SWS; ↑ REM
			AAV_9-EF1α-DIO-hM4Di-mCherry	0.5 mg/kg	↑ Wake; ↓ SWS; ↓ REM
Basal ganglia					
Yuan et al. (2017) eLife	Caudal STRd (iMSN)	*Adora2a-Cre* (B6) mice	AAV-hSyn-DIO-hM3Dq-mCherry	1 mg/kg	↓ Wake; ↑ SWS; ↔ REM
	Centromedial STRd (iMSN)	*Adora2a-Cre* (B6) mice	AAV-hSyn-DIO-hM3Dq-mCherry	1 mg/kg	↓ Wake; ↑ SWS; ↔ REM
	Centrolateral STRd (iMSN)	*Adora2a-Cre* (B6) mice	AAV-hSyn-DIO-hM3Dq-mCherry	1 mg/kg	↓ Wake; ↑ SWS; ↔ REM
	Caudal STRd (iMSN)	*Adora2a-Cre* (B6) mice	AAV-hSyn-DIO-hM3Dq-mCherry	1 mg/kg	No effect
	GPe (PV)	*Pvalb-Cre* (129) mice	AAV-hSyn-DIO-hM4Di-mCherry	1 mg/kg	↓ Wake; ↑ SWS; ↔ REM
	STRd (iMSN) → GPe (PV)	*Adora2a-Cre/Pvalb-Cre* (129/B6) mice	AAV-hSyn-DIO-hM3Dq-mCherry (in STRd) and	1 mg/kg	No effect

(continued)

Table 1 (continued)

Authors	Brain area	Animal model[a]	DREADD-containing vector[b]	CNO dose	Effects on vigilance states[c]
			AAV-EF1α-Flex-taCasp3-TEVp + AAV EF1α-DIO-eGFP (in GPe: ablation)		
	STRd (iMSN)	*Adora2a-Cre* (B6) mice	AAV-hSyn-DIO-hM4Di-mCherry	1 mg/kg	↑ Wake; ↓ SWS; ↓ REM
Oishi et al. (2017) Nat Commun	NAc (iMSN)	*Adora2a-Cre* (B6) mice	AAV_{10}-hSyn-DIO-hM3Dq-mCherry	0.01–1 mg/kg	↓ Locomotor activity; ↑ SWS
			AAV_{10}-hSyn-DIO-hM4Di-mCherry	0.03–0.3 mg/kg	↓ SWS
Limbic system					
Hasegawa et al. (2017) Proc Natl Acad Sci U S A	Amygdala (LA, BLA, CeA)	*Orexin/ataxin-3* (B6) mice	$AAV_{2/rh10}$-SynI-iCre + $AAV_{2/rh10}$-EF1α-DIO-hM3Dq-mCherry	5 mg/kg	↑ Cataplexy
			$AAV_{2/rh10}$-SynI-iCre + $AAV_{2/rh10}$-EF1α-DIO-hM4Di-mCherry	5 mg/kg	↓ Cataplexy

BLA basolateral amygdala, *BNST* bed nucleus of the stria terminalis, *CeA* central amygdala, *dDpMe* dorsal part of the deep mesencephalic nucleus, *GPe* globus pallidus, external part, *HDB* horizontal limb of the diagonal band, *LA* lateral amygdala, *LC* locus coeruleus, *LH* lateral hypothalamus, *LPO* lateral preoptic area, *MCH* melanin-concentrating hormone, *MCPO* magnocellular preoptic nucleus, *MnPO* median preoptic nucleus, *NAc* nucleus accumbens, *PB* parabrachial nuclei, *PBel* external lateral parabrachial nucleus, *PPT* pedunculopontine tegmentum, *PZ* parafacial zone, *RMV* rostral ventromedial medulla, *SI* substantia innominate, *SNc* substantia nigra, pars compacta, *pSOM* ventromedial medulla rostral to the inferior olive, *STRd* dorsal striatum, *SUM* supramammillary nucleus, *TMN* tuberomammillary nucleus, *VP* ventral pallidum, *VTA* ventral tegmental area, *ZI* zona incerta

[a]Genetic background or strain mentioned when available

[b]Serotype and promoter used for AAV constructs provided when available

[c]Main effects are reported, otherwise mentioned wake, SWS and REM refer to total wake SWS and REM sleep amounts

and lowering the level of DREADD expression using diluted viral preparations or using a weaker promoter should be considered.

Another conceptual issue relates to the fact the GPCRs can activate more than one G-protein isoform and can also signal through G-protein-independent pathways involving β-arrestin (see for review Roth 2016). Thus, it questions under which conditions β-arrestin signaling is activated and whether the effects observed on neuronal activity and behavior result from canonical or noncanonical (G-protein-independent) signaling. To date, there have been no reports suggesting that the actions of CNO-mediated neuronal silencing or excitation, through hM4DGi and hM3DGq, respectively, could be explained by other mechanisms than altered neuronal firing. Without excluding alternative pathways, the argument developed in favor of major canonical signaling is based on several studies applying optogenetics and DREADDs on the same neuronal populations that globally show equivalent effects in nature and magnitude. However, some differences between optogenetics and DREADD studies are still observed. This is notably the case when manipulating hypothalamic peptidergic systems such as hypocretin/orexin (Hcrt/Orx)- or melanin-concentrating hormone (MCH)-containing neurons and examining their impact on sleep macroarchitecture (see Sect. 3.5). While optogenetic stimulation of Hcrt/Orx neurons induces sleep fragmentation without affecting global sleep or wake amounts (Rolls et al. 2011; Bonnavion et al. 2015), DREADD activation results in prolonged wake and sleep deprivation over several hours (Sasaki et al. 2011). Many factors here can have an impact underlying such differences including viral transduction versus transgenic mice, or photostimulation paradigms versus CNO-driven activation, whose impact on Hcrt/Orx neuronal firing in vivo is unknown. Still an additional factor to consider could relate to the activation of differential downstream effector pathways and physiological changes that could favor multiple transmitters' co-release (see for review Bonnavion et al. 2016; Schöne and Burdakov 2017). Interestingly, a DREADD that signals exclusively via β-arrestin has been developed (Nakajima and Wess 2012) allowing to tackle in vivo the specific behaviors downstream of β-arrestin signaling.

One last concern with GPCR inherent properties relates to possible desensitization and subsequent DREADD receptor downregulation following repeated administration of CNO. As with many GPCRs undergoing significant downregulation only following hours of agonist treatment, chronic treatment with CNO might result in diminished responses or might induce a shift in the dose-response curve to higher concentrations due to DREADD receptor desensitization and internalization. However, the degree of desensitization greatly depends on the extent to which receptors are expressed. When DREADD expression is quite high, lower concentrations of the chemical actuator are needed to achieve a maximal response (see for review Roth 2016). Therefore, considering the very high levels and density of virally or transgenically induced DREADD expression, it is likely that the cellular responses will be less sensitive to repeated CNO than when the mutated receptors are expressed at lower levels. So far, no significant desensitization was seen or reported using viral or transgenic approaches.

2.3 The CNO Versus Clozapine Case

Before the expansion of the CNO/DREADD system use, former studies focusing on clozapine metabolism showed that clozapine is converted into its N-oxide metabolite CNO but that a reversible pathway exists for a small fraction of CNO back-metabolized to clozapine in guinea pigs and humans (Jann et al. 1994; Chang et al. 1998). From the start, this observation challenged the mechanisms of action of CNO in vivo and questioned whether this conversion could also occur in other species including rodents and nonhuman primates. From many reports, CNO appeared to be pharmacologically and behaviorally inert in rodents (Bender et al. 1994; Armbruster et al. 2007; Alexander et al. 2009; Guettier et al. 2009). However, recent studies performed in mice, rats, and monkeys further investigated this possibility by measuring the presence of clozapine following CNO administration through various assays (Hellman et al. 2016; Gomez et al. 2017; Raper et al. 2017). Additionally, the blood-brain permeability of CNO has been reexamined (Hellman et al. 2016; Ji et al. 2016; Nagai et al. 2016; Gomez et al. 2017; Raper et al. 2017). These recent papers emphasize three major points: (1) the affinity of clozapine to bind DREADDs is higher than CNO (Gomez et al. 2017); (2) CNO may not cross the blood-brain barrier (Hellman et al. 2016; Gomez et al. 2017; Raper et al. 2017); and (3) CNO can be converted to clozapine under certain doses in monkeys and rodents (Hellman et al. 2016; Gomez et al. 2017; Raper et al. 2017).

The first point was made based on binding data and suggests that clozapine could be more potent than CNO in activating DREADDs. Still, several studies showed that CNO efficiently and rapidly controls neuronal firing both in vitro and in vivo (Alexander et al. 2009; Krashes et al. 2011; Parnaudeau et al. 2013; Alcacer et al. 2017). Importantly, it is also possible that clozapine, even at very low doses, can activate endogenous receptors in addition to its action on DREADDs. Thus, to appropriately compare efficiencies and kinetics of clozapine and CNO on targeted neurons and neighbor cells, both drugs should be examined in parallel with in vitro and/or in vivo electrophysiological recordings. In addition, Gomez and colleagues performed locomotor behavioral tests to examine the effects of both CNO and "sub-threshold concentrations of clozapine in dopamine D1 receptor-Cre mice expressing hM4DGi transgene in the striatum and control GFP mice (Gomez et al. 2017). Their results suggest that 1 mg/kg CNO would be equivalent to 0.01 mg/kg clozapine to control locomotion in striatal D1-hM4Di-expressing mice without affecting locomotion in control animals. However, the lack of locomotor effects in control mice treated with sub-threshold concentrations of clozapine does not imply that it would not affect other behaviors or cognitive processes. Plus, this efficiency on DREADD-transfected neurons is limited to one neural system for now and should be further tested over DREADD-validated ones on various behavioral responses. The need for developing alternative compounds appears critical, but the switch to clozapine should be carefully considered.

To conclude the last two other points, Gomez and colleagues first employed PET imaging and autoradiography in rats and mice that shows low-to-no signal of radiolabeled CNO in brain tissue, which is contrasting with similar experiments

also performed in transgenic DREADD mice showing a highest contrast of radio signals for DREADD versus background with CNO than with clozapine (Ji et al. 2016). The major argument resides in the fact that the radiolabeled CNO signal could have been due to converted radiolabeled clozapine. Indeed, contrasting results from clozapine/CNO plasma measurements were reported in rodents and in monkeys detecting clozapine after CNO systemic injections (Hellman et al. 2016; Gomez et al. 2017; Raper et al. 2017) or not (Alexander et al. 2009; Guettier et al. 2009; Nagai et al. 2016). It still remains elusive to explain or reconcile these differences. Importantly, if such conversion happens, it primarily occurs in the liver through cytochrome P450 enzymes, which are also present in the brain but at very low levels (Mahler and Aston-Jones 2018). In addition, several studies demonstrated that CNO efficiently activates signaling pathways on cell cultures and on diverse mammalian and drosophila neuronal systems, in a shorter time frame than what is observed for CNO/clozapine conversion (see for review Roth 2016). Indeed, Gomez and colleagues reported that clozapine metabolized from CNO accumulates over time such that its effects may be strongest 2–3 h after CNO injection. Based on the short plasma half-life of CNO within 2 h (Alexander et al. 2009; Guettier et al. 2009), most studies so far examined its effects within this first 2-h period. However, late-onset effects on behavior were not necessarily analyzed outside this 2-h window and should be further monitored from now on.

It becomes clear that CNO is not an inert ligand upon the dose used. “Safe” doses to administer in a systemic manner range from 0.1 to a maximum of 3 mg/kg in order to avoid or minimize this interconversion. Also, in light of the low distribution in the central nervous system of CNO, its delivery route could be revised using local intracerebral (i.c.) injections (Stachniak et al. 2014; Vazey and Aston-Jones 2014) instead of systemic administration to enhance its actions locally but involves a more invasive approach.

Finally the critical point that these studies highlighted is the importance of controlling and comparing CNO effects in appropriate DREADD-free control animals, in addition to vehicle injections in the same DREADD-expressing subjects. Ultimately these comparisons within and between subjects will reinforce the significance and specificity of action of CNO. Indeed most studies limited their results comparing CNO to vehicle/saline injections, while growing evidences from mice, rats, and monkeys studies report endogenous effects of CNO at certain doses in control DREADD-free animals (Eldridge et al. 2015; MacLaren et al. 2016; Gomez et al. 2017; Varin et al. 2018). Taken the possible conversion of CNO and the sedative properties of clozapine (Hinze-Selch et al. 1997), these controls are crucial in sleep studies. Indeed, a recent study using DREADD approaches to control and modulate hypothalamic MCH neurons across the sleep-wake cycle showed that CNO at the doses of 5 and 10 mg/kg increases slow-wave sleep and inhibits REM sleep in control wild-type mice (Varin et al. 2018).

Altogether, these recent findings calling to re-evaluate the use of CNO at certain doses through a systemic delivery route stress the need to develop and test alternative compounds. Interestingly, non-CNO chemical actuators for DREADDs have been tested such as a newly developed actuator named compound 21, and the

FDA-approved hypnotic compound perlapine, both highly selective for hM3DGq (Chen et al. 2015). Importantly, compound 21 is not back-metabolized via normal routes to clozapine or related compound and thus represents a good alternative to CNO (Chen et al. 2015). Perlapine has a modest affinity for certain biogenic amine receptors such as 5-HT2A, 5-HT6, 5-HT7 and D4, but appears to have more than a 10,000-fold selectivity for activating hM3DGq versus muscarinic receptors (Chen et al. 2015). With careful titration to define sub-threshold doses, application of perlapine in conjunction with DREADD-targeted activation could still be quite relevant to study sleep-wake circuits notably in models of insomnia, and sedative processes.

3 CNO-DREADD Experiments in Sleep Studies

The following sections review the major findings identifying the role of defined cell populations in the regulation of the sleep-wake cycle that emerged from pharmacosynthetic approaches in rodents over the past 10 years (Fig. 1). So far, the techniques employed appear to be exclusive to the use of the CNO/DREADD system.

Following up on the later concerns about the use of CNO and its possible conversion to clozapine, most of the sleep studies presented here used CNO delivered systemically under the critical dose of 3 mg/kg. Although the majority of the literature compared CNO effects to vehicle injections on their main figures, most studies examined whether CNO at the dose employed had any endogenous effects on DREADD-free control animals. So far in sleep studies, only one study explicitly reported undesired sleep effects from CNO injections in wild-type mice at the dose of 5 and 10 mg/kg (Varin et al. 2018). To have a clear vision of the procedures employed, we drew up a table integrating all the studies presented here summarizing the neuronal system and brain structure targeted, viral or transgenic approaches, CNO dose used, and the major effects found on sleep-wake states (see Table 1) (Fig. 1).

3.1 GABA Systems

For the last decade, the use of both optogenetic and DREADD techniques highlighted the multifaceted role of the major inhibitory neurotransmitter GABA in sleep and wakefulness processes. Although the role of GABA in sleep induction and maintenance is well established since most of the hypnotic treatments targets $GABA_A$ receptors (Watson et al. 2012), the identification of the GABAergic populations involved remained elusive. Part of the reasons come from the fact that GABAergic neurons are often intertwined among other neurons utilizing different neurotransmitters but also that several neuronal populations previously thought to release only glutamate (Glu), ACh, dopamine (DA), or histamine (His) also release GABA (see for review Tritsch et al. 2016). These complex configurations prevented the access and possibility to question the respective function and physiological role of GABA neurotransmission until transgenic animals and gene targeting techniques

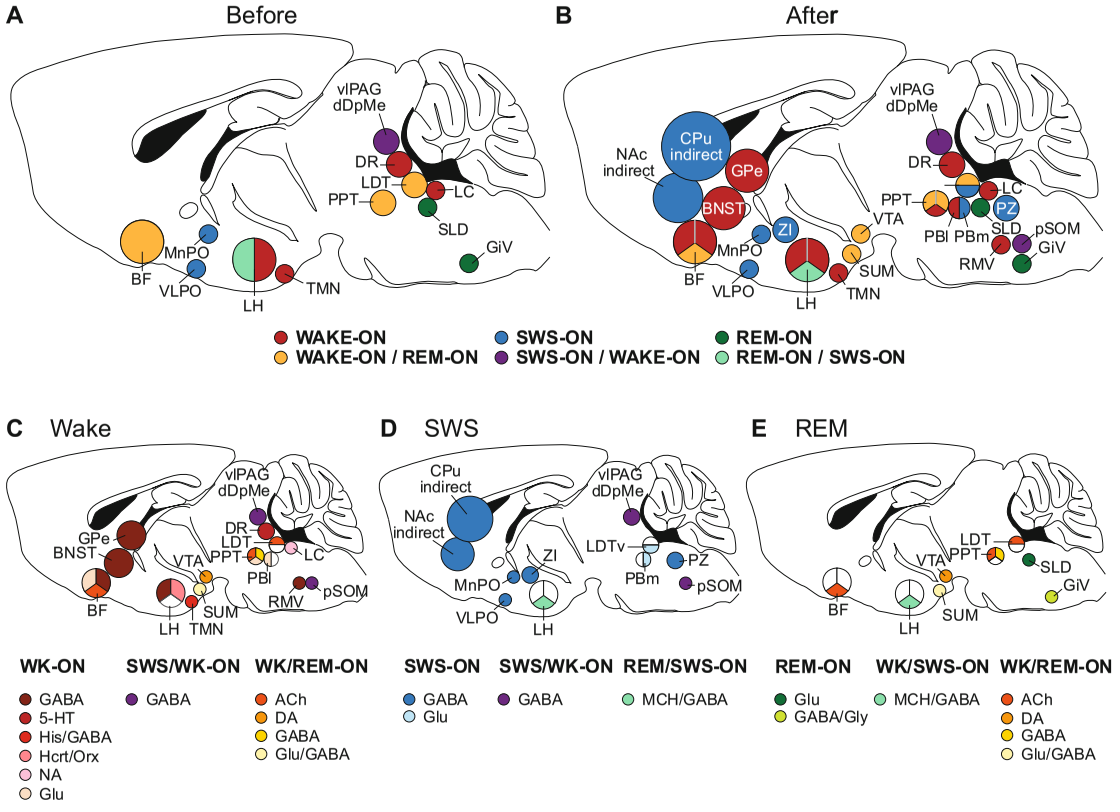

Fig. 1 Schematic summary of sleep-/wake-related structures evidenced by chemogenetics. Here are displayed most of the structures involved in brain state transitions between waking and sleeping states before (**a**) and after (**b**) chemogenetic applications. Wake-, SWS-, and REM sleep-promoting nuclei originating from the classical model of sleep/wake regulation (see for reviews Brown et al. 2012 and Luppi et al. 2017) and chemogenetically identified or refined structures are subdivided between specific state-promoting profiles (wake, red; SWS, blue; REM sleep, green) and multiple state-promoting profiles such as wake- and REM sleep-promoting systems (orange), wake- and SWS-associated nuclei (purple), and SWS- and REM sleep-related neurons (teal). In (**c**–**e**), nuclei associated with either wake (**c**), SWS (**d**), or REM sleep (**e**) are depicted in regard to their neurochemical nature labeled with individual color code

including optogenetic and DREADD were developed. Thanks to these approaches, our understanding of sleep circuits integrating multiple GABAergic systems has been considerably revised for a predominant role of various GABA neurons in the control of the sleep-wake cycle (see for reviews Brown and McKenna 2015; Luppi et al. 2017) (Fig. 1). We review here the different studies using DREADD approaches on various GABAergic populations that evidenced a role for GABA neurotransmission in the enhancement and maintenance of both sleep and wake states (Fig. 1).

3.1.1 GABAergic Sleep-Promoting Neurons of the Preoptic Area

A large body of evidence indicates that GABAergic neurons of the preoptic area (POA) of the anterior hypothalamus play a crucial role in slow-wave sleep (SWS) (Luppi et al. 2017). Indeed the CNO-induced excitation of GABAergic neurons in the whole preoptic area resulted in an increase in SWS amounts during both the diurnal inactive and the nocturnal active photoperiods (Saito et al. 2013). More specifically, a dense population of SWS-active GABAergic neurons were found in the median (MnPO) and the ventrolateral (VLPO) preoptic nuclei, evidenced by a high number of cFos-positive neurons (immediate early gene used as a marker of neuronal activation) correlating with time spent in SWS (Luppi et al. 2017). However, the causal role of these different populations in the MnPO and VLPO in the initiation and maintenance of SWS still needed to be confirmed. Besides, the POA is composed of a mixture of mostly GABAergic sleep-active, wake-active, and state-indifferent neurons (Takahashi et al. 2009). Interestingly, a group of researchers took advantage of this consistent cFos pattern in the POA elicited by sleep enhancement following either total sleep deprivation or sedation, to control the activity of these sleep-active neurons and test their causal role in sedation and sleep (Zhang et al. 2015). To do so, Zhang and colleagues employed a Fos-TRAP/TetTag-DREADD approach. This approach consists in the expression of a cFos-promoter inducible hM3DGq gene selectively in the POA, MnPO, or lateral POA (LPO), by TetTagging (Reijmers et al. 2007). More specifically, hM3DGq gene is only turned on following cFos expression induced by neuronal activation that in turn drives tTA expression. Temporal control over tTA-TRE recombination, and thus cFos-dependency, is achieved through doxycycline administration and removal. As a result, neurons that have been activated in vivo by a stimulus are tagged with hM3DGq and can be specifically reactivated later by systemic CNO injection. To examine the causal relationship between these neurons and sleep, TetTagging is performed during recovery sleep following total sleep deprivation or during sedation induced by the adrenergic α2 agonist, dexmedetomidine. Importantly, reactivating either sleep rebound-tagged or sedation-tagged neuronal populations produces sustained SWS, 20 min after CNO injections, together with a lower body temperature suggesting that α2 adrenergic-induced sedation and recovery sleep share homologous hypothalamic circuitry (Zhang et al. 2015). Single-cell RT-qPCR revealed that a majority of TetTagged POA neurons are GABAergic (84%) and the remaining cells glutamatergic. This study brings the proof of concept that some MnPO and LPO GABAergic neurons are not only passive sleep-active neurons but are indeed major effectors in SWS induction. In another study, downstream targets of POA

GABAergic neurons were further examined with optogenetics highlighting wake-active histaminergic (His) neurons of the tuberomammillary nucleus (TMN) as a major target to inhibit in order to elicit SWS (Chung et al. 2017). In agreement with this observation, acute optogenetic silencing of TMN His neurons rapidly induces SWS (Fujita et al. 2017).

The next three discoveries using DREADDs on GABAergic neurons allowed to identify three novel systems involved in the promotion or facilitation of sleep in mice.

3.1.2 GABAergic Sleep-Promoting Neurons in the Rostral Medulla

Early experiments 60 years ago suggested that the caudal brainstem contains SWS-promoting system (see for review Anaclet and Fuller 2017). While most studies focused on the rostral hypothalamus and in particular the POA as a major structure at the core of sleep onset and maintenance mechanisms since von Economo's first observations (Economo 1930), seminal work proposing the hypothesis of a brainstem sleep system was dismissed until recently and further examined with DREADDs. The newly identified neuronal ensemble located lateral and dorsal to the facial nerve in the rostral medullary brainstem, named the parafacial zone (PZ), was first evidenced by a state-specific cFos pattern related to sleep (Anaclet et al. 2012). Those sleep-active neurons express the vesicular GABA/glycine transporter (VGAT) also known as vesicular inhibitory amino acid transporter (VIAAT). Using transgenic *VGAT-cre* mice in combination with cre-dependent viral vector carrying DREADDs, Anaclet and colleagues challenged both the sufficiency and necessity of PZ GABAergic neurons to control sleep-wake states (Anaclet et al. 2014). Upon CNO-mediated activation of hM3DGq-expressing GABAergic neurons in the PZ, animals quickly express a predominance of SWS state within the first hour during their nocturnal active period, lasting for 3 h. This increase of SWS was observed at the expense of both wakefulness and REM sleep and is associated with increased cortical slow-wave activity (Anaclet et al. 2014). Reciprocally, CNO-induced inhibition of hM4DGi-expressing PZ neurons during the inactive diurnal period rapidly and strongly decreases SWS (Anaclet et al. 2014). One puzzling result harvested in this study is the consistent inhibition of REM sleep observed following both the activation and the inhibition of PZ GABAergic neurons. This effect remains unclear and could depend on parallel circuits impacting on REM sleep initiation and its EEG features. However this effect coupled to the absence of REM sleep rebound also questions the maintenance of a natural sleep physiological regulation after CNO-induced activation or inhibition of PZ neurons.

Using optogenetic-mediated circuit mapping, Anaclet and colleagues dissected the circuitry underlying PZ GABAergic promotion of SWS. They observed that neurons in the parabrachial nucleus (PB) projecting to the magnocellular basal forebrain (BF) receive monosynaptic inhibitory inputs from GABAergic PZ neurons and that BF neurons projecting to the dorsomedial prefrontal cortex (dmPFC) receive monosynaptic excitatory inputs from glutamatergic PB neurons (Anaclet et al. 2014). Previous work performed by the same group also demonstrated that the PB to BF pathway is critical for the desynchronization of the cortical EEG (Fuller

et al. 2011). This follow-up study shows a direct inhibitory control of PZ VGAT neurons onto PB neurons projecting to the BF (Anaclet et al. 2014). Thus, the authors hypothesize that the PB inhibition applied by PZ neurons would decrease the major excitatory inputs to dmPFC-projecting neurons in the BF that would in turn modulate cortical activity and result in disrupted arousal. This brainstem-forebrain-cortex pathway involving medullary release of GABA would act in parallel to the reticulo-thalamo-cortical pathway (Llinas and Steriade 2006) to control behavioral and electrocortical arousal. However the causal relationship of this series of inhibitions and activations of different brain structures and neuronal subpopulations occurring cooperatively to control behavioral arousal remains to be established.

3.1.3 Subthalamic GABAergic Control of Sleep

Another study investigating the organizational complexity of the diencephalon highlighted a subset of GABAergic neurons located in the ventral zona incerta (ZI) in the control of sleep (Liu et al. 2017). This subset of GABAergic neurons is characterized by the expression of a LIM homeodomain transcription factor gene, Lhx6, early in development. Using a Lhx6-cre transgenic mice, the researchers confirmed the presence of this marker in GABAergic neurons co-expressing GAD1 and VGAT, distinct from Hcrt/Orx and MCH neurons, in a zone extending from the ventral ZI through the dorsomedial (DMH) and lateral (LH) hypothalamus to the posterior hypothalamus (Liu et al. 2017). The first link associating ZI Lhx6/GABAergic neurons activity with sleep was shown by the circadian distribution of cFos staining in ZI Lhx6 neurons with increased cFos levels correlating with circadian times associated with high sleep pressure (Liu et al. 2017). However, puzzling results were obtained on cFos levels after a 6-h sleep deprivation alone or in combination with 1 h of recovery sleep. Both conditions elevate cFos in ZI Lhx6 neurons in a similar manner, which does not discriminate whether cFos pattern reflects purely wake- or sleep-active neurons or neurons integrating sleep pressure (Liu et al. 2017). Using DREADD, Liu and colleagues directly assessed whether the manipulation of ZI Lh6x GABAergic neuron signaling modulates sleep. In vivo, the selective activation of hM3DGq or hM4DGi receptors in ZI Lh6x neurons affects SWS amounts in a bidirectional manner, promoting or reducing SWS, respectively (Liu et al. 2017). However these effects are observed from 4 to 6 h after CNO injection during the diurnal inactive period. These late-onset effects are surprising and could be interpreted as unspecific or attributed to CNO conversion to clozapine (see Sect. 2.3). However, the CNO dose used here is among the lowest (0.5 mg/kg) which would dismiss any major effects of late interconversion of CNO to clozapine. In addition, CNO injection in control DREADD-free animals was reported to be inert. More immediate effects are observed when CNO is delivered during the active nocturnal period enhancing SWS with increased delta power. However, inconsistent results were found on REM sleep: no effect was observed when CNO was injected during the active phase, while a bimodal effect occurred when CNO was administered during the inactive period characterized by an immediate inhibition

followed by long-lasting enhancement of REM sleep. Further connectivity studies would be necessary to interpret these effects on REM sleep.

An alternative way to interpret these results would be that ZI Lh6x neuron activation is related to the integration of sleep pressure and sleep need. This would explain the late-onset SWS-promoting effects observed after neuronal excitation as well as the absence of increase in SWS amounts in the second half of the diurnal inactive period, after the inhibition of Lhx6 neurons. Experiments involving total sleep deprivation and inhibition of ZI Lh6x neurons during sleep deprivation or immediately in the beginning of the recovery period would better address this view. Moreover, mapping experiments show that ZI Lh6x neurons form an interconnected local network, which could partly explain slow-onset for SWS enhancement, implying that other ZI GABAergic neurons would be involved in either wake-promoting processes or REM suppression (Liu et al. 2017). However, another study showed that global DREADD activation of ZI VGAT-expressing neurons does not exhibit any increase in wakefulness following CNO injections at 0.3 mg/kg (Venner et al. 2016).

Tracing experiments indicated that ZI Lh6x neurons receive inputs from various brain structures both unrelated and related to the control of sleep-wake cycle (Liu et al. 2017). Among these structures, Liu and colleagues focused on reciprocal connections that ZI Lh6x neurons share with the lateral hypothalamus (LH). They observed that both GAD2-expressing GABAergic cells and Hcrt/Orx neurons of the LH are directly inhibited after ZI Lh6x optogenetic activation (Liu et al. 2017). As a result, they hypothesized that part of the mechanisms involved in ZI Lh6x-driven enhancement of sleep requires inhibition of the wake-promoting neurons of the LH. Consistently, direct inhibition of a subpopulation of GABAergic (VGAT-expressing) cells in the LH induces SWS, mainly through a disinhibition of the thalamic reticular nucleus (Herrera et al. 2015). Moreover, inhibition of LH GAD2-expressing neurons reduces physical activity and locomotion (Kosse et al. 2017). However, overall, modest effects result from ZI Lh6x DREADD activation considering that CNO-induced enhancement of sleep occurs with delay and is limited to increased amounts but unchanged SWS bout mean duration or wake-to-sleep transitions. Thus, it is unlikely that ZI Lh6x GABAergic neurons act as primary effectors in SWS induction as POA or PZ neurons do.

To apprehend the complexity of diencephalic and mesencephalic neuronal networks regulating sleep-wake cycles, modeling approaches could further inform on the hierarchical organization of these parallel circuits and their dynamics and could in particular better identify and position the role of ZI Lh6x GABAergic neurons and their relative involvement in sleep promotion of sleep pressure mechanisms. Besides, further investigations are necessary to track down functional diversity among ZI GABAergic neurons.

3.1.4 Sleep Control in the Basal Ganglia

At last, by investigating the mechanisms by which adenosine promotes sleep (Bjorness and Greene 2009), two groups of researchers recently identified alternative pathways in the basal ganglia (BG) circuitry necessary and sufficient to generate

slow-wave activity and a behavioral state resembling sleep using DREADDs (Oishi et al. 2017; Yuan et al. 2017). The BG is composed of four major nuclei including the striatum (caudate and putamen, CPu), which is the primary input nucleus, globus pallidus (GP), subthalamic nucleus (STN), and substantia nigra pars reticulata (SNr) as the major output nucleus. The BG is strongly interconnected with the cortex, thalamus, and midbrain structures including the DA-enriched ventral tegmental area (VTA) and substantia nigra pars compacta (SNc), as well as many other brain areas from the limbic system (Graybiel 2008). The striatum is divided into three territories, a dorsolateral (DLS), a dorsomedial (DMS), and a ventral domain also named nucleus accumbens (NAc), associated with distinct functions in regard to their cortical and limbic inputs (Graybiel 2008). These subdivisions, topographically connected with cortical and subcortical structures, carry information related to sensorimotor, associative, and limbic functions, respectively. The striatum is seen as a hub orchestrating BG functions including action control, skill learning, habit formation, incentive motivation, and goal-directed behaviors (Graybiel 2008; Balleine and O'Doherty 2010; Jin and Costa 2010). The BG activity during sleep-wake states has been largely underexplored, while dysfunction of the striatum is frequently associated with sleep disturbances (see for review Lazarus et al. 2012). Some studies indicated two major points: (1) several distinct firing patterns in BG nuclei across the sleep-wake cycle (Magill et al. 2000; Urbain et al. 2000) and (2) activity changes in striatal neurons from cyclic, fast active firing to highly irregular firing during sleep-to-wake transition (Mahon et al. 2006), suggesting distinct striatal processing depending on arousal state. In addition, the striatum is composed predominantly of GABAergic medium spiny output neurons (MSNs) divided into two populations: those expressing dopamine D_1-receptors, directly projecting to the globus pallidus pars interna (GPi)/SNr, and those expressing adenosine A_{2A} and dopamine D_2 receptors, projecting to the globus pallidus pars externa (GPe) involving multiple nodes down to the SNr (Durieux et al. 2011). These two neuronal subpopulations form the "direct" (dMSNs) and "indirect" (iMSNs) pathways of the BG thought to act concomitantly but antagonistically during action selection to execute appropriate motor and cognitive responses (see for reviews Balleine and O'Doherty 2010; Jin and Costa 2010). In this context, the recent studies using DREADDs to tackle the role of A_{2A}-expressing iMSNs, known as action suppressors, in the control of vigilance states further challenge the existence of parallel sleep-wake regulatory pathways and question the function of BG in sleep as well as the role of the hypnogenic factor adenosine (Oishi et al. 2017; Yuan et al. 2017).

Using hM3DGq and hM4DGi cre-dependent viral vectors targeting A2A-Cre-expressing iMSNs in the striatum, two groups of researchers observed that DREADD control of iMSNs located in the core region of the NAc (Oishi et al. 2017) and in the dorsal striatum in its rostral extension (Yuan et al. 2017) influences sleep in a bidirectional manner. DREADD hM3DGq activation of iMSNs in both striatal regions results in the promotion of a state resembling SWS, characterized by slow and high-voltage EEG. Reciprocally, hM4DGi-dependent inhibition of iMSNs reduces SWS amounts. Previous work examining the effects of local infusion of A_{2A}

agonist showed that adenosine-mediated activation in the ventral striatum elicits both SWS and REM sleep in rats (Satoh et al. 2006). However, here, DREADD hM3DGq activation of both ventral and dorsal iMSNs promotes SWS without affecting REM sleep amounts. Subtle differences are also observed between the two striatal subdivisions on SWS facilitation evoked by A_{2A}-iMSNs activation, which is underlined by an increase in the number of SWS episodes with NAc core activation (Oishi et al. 2017) and, on the other hand, by a lengthening of SWS episodes with rostro-dorsal activation (Yuan et al. 2017).

Both DREADD-driven effects on sleep increase are recapitulated by optogenetic stimulation of iMSNs in the NAc core (Oishi et al. 2017) or stimulation of dorsal iMSN terminals in the GPe (Yuan et al. 2017). However, kinetics of effects between optogenetics and DREADD activation importantly differ when targeting NAc core iMSNs (Oishi et al. 2017). The optogenetic-induced sleep-like state is almost immediate, while CNO-promoting effects appear only in the second hour after i.p. injection. In comparison, sleep induction resulting from hM3DGq activation of PZ neurons occurs rapidly after 15–20 min following injection for a similar dose of CNO (Anaclet et al. 2014; Oishi et al. 2017). This comparison cannot be made with DREADD activation of dorsal iMSNs as the dose of CNO employed was higher. Importantly, CNO-mediated activation of hM3DGq-expressing iMSNs was found to increase the response to depolarizing stimuli but does not induce any spiking per se (Alcacer et al. 2017). Unlike optogenetics (Oishi et al. 2017), DREADD activation of iMSNs through hM3DGq pathway likely increases the neuronal excitability but does not excite neurons, which still requires further in vivo confirmations. However, it might explain the differences in CNO versus optogenetic kinetics. In contrast, DREADD-dependent Gi inhibition of NAc core iMSNs seems to induce a reduction of SWS amounts more rapidly within the first hour following i.p. injection (Oishi et al. 2017). Interestingly in both regions, NAc core and rostro-dorsal striatum, presumed inhibition of hM4DGi-expressing iMSNs decreases SWS amounts for at least 4 h without inducing (Oishi et al. 2017; Yuan et al. 2017) or affecting homeostatic sleep rebound after sleep deprivation (Oishi et al. 2017), suggesting that rebound mechanisms may involve different pathways.

As a result, the involvement of the striatal indirect pathway in sleep induction is puzzling. Oishi and colleagues proposed that NAc iMSNs would act ahead of POA sleep-active neurons upon the influence of adenosine levels, by depressing the arousal circuitry in BF, which, in turn, would release POA sleep-promoting system. On the other hand, Yuan and colleagues were also able to promote SWS by directly inhibiting with DREADD the downstream target neurons of dorsal iMSNs in the GPe that are also inhibitory cells expressing parvalbumin (PV). Moreover, the selective lesion of PV neurons in the GPe abolished the increase in SWS caused by DREADD activation of iMSNs (Yuan et al. 2017), suggesting that the activation of A_{2A}R neurons in the striatum inhibits GPe PV neurons to promote SWS. These alternative circuits emerging from the striatum require further investigations notably including in vivo monitoring of iMSNs activity across the natural sleep-wake cycle in addition to tracing studies from the NAc core and GPe to further support their role in SWS induction. Interestingly, sleep-wake cycle is not altered by photostimulation

of iMSNs in the shell region of the NAc (Oishi et al. 2017) or by DREADD activation of the caudal part of the dorsal striatum (Yuan et al. 2017), suggesting specialized functions in the different striatal territories for the modulation of vigilance states.

It is important to keep in mind that the primary functions of the striatum are critical for behaving animals and thus arousal, in order to process multiple signals and execute motor control and action selection. Even if it does not necessarily imply that these neurons cannot have a role in sleep, the papers mentioned above focused their analysis on sleep-related parameters. However sleep and wake patterns are organized in a continuum and should not be considered as segregated events. The DREADD activation of NAc core iMSNs induces a concomitant reduction of locomotor activity (Oishi et al. 2017), but wakefulness amounts and waking EEG pattern have not been reported to further inform on mice levels of arousal or alertness. Reciprocally, DREADD inhibition of the NAc indirect pathway was found to increase ambulatory activity (Carvalho Poyraz et al. 2016), and DREADD inhibition of iMSNs in the dorsal striatum is only efficient in increasing wake during the active dark period (Yuan et al. 2017). However, activation of the latter neurons is also accompanied by decreased wake amounts with shortened mean duration (Yuan et al. 2017). This mixture of behaviors is critical when the dorsal striatopallidal pathway is involved as its activation through DREADD was also found to control general motor output (Alcacer et al. 2017). Therefore, it is still questionable whether the effects observed here reflect SWS induction or loss of motor control progressively driving the animals to enter into an immobile quiet state of wakefulness harder to distinguish from sleep. Alternatively, the effects described above could depend on an inhibition of wake maintenance mechanisms, which would ultimately and indirectly facilitate sleep onset. For future investigations, it is capital to examine closely iMSNs activity from distinct striatal subdivisions during spontaneous sleep-wake states to better characterize a potential role in sleep promotion and wake maintenance and challenge the influences of the direct pathway by opposition. In addition to the deconstruction of state transitions, it would be interesting to address the functions of BG in sleep that could be relevant to learning and memory processes.

3.1.5 GABA: Not Just a SWS Driver

GABA was first considered to act as the principle driver for SWS by silencing waking systems. However, recent findings using optogenetics and DREADD highlighted a more complex role for GABA neurotransmission in all vigilance states. DREADD approaches contributed to distinguish two types of GABA cells with the primary function to either promote waking or repress REM sleep, located in the hypothalamus, in the BF and cortex, or in the brainstem, respectively (see for review Luppi et al. 2017). The contributions of GABA from the BF in various features of waking will be addressed in a following section reviewing the role of ACh (see Sect. 3.3.2). Cortical GABAergic interneurons, principally PV neurons, have been shown to exert a critical role in the generation of gamma rhythm during waking, which was mainly investigated and recapitulated with optogenetics (see Adamantidis and Lüthi 2018). We will thus review here three papers using DREADDs highlighting the

diverse role of GABA in different brain regions (Fig. 1): promoting wake through the hypothalamus in the LH (Venner et al. 2016) and in the bed nucleus of the stria terminalis (BNST) (Kodani et al. 2017) and suppressing REM sleep in the ventromedial medulla (Chen et al. 2017).

Wake-Promoting GABAergic Neurons in the Hypothalamus

Optogenetics identified for the first time a subpopulation of GABAergic neurons in the LH, targeted by the expression of VGAT transporter in a *VGAT-IRES-Cre* line, responsible for a rapid switch from SWS to waking state involving a direct control of the thalamic reticular nucleus (TRN) as well as on the locus coeruleus and the periventricular thalamus (Herrera et al. 2015). Interestingly, using DREADDs in a similar transgenic *VGAT-IRES-Cre* mouse, Venner and colleagues described a GABAergic neuronal subpopulation in the LH qualitatively displaying a similar projection pattern in the POA and the pontine tegmentum but lacking axonal innervation of the TRN (Venner et al. 2016). Some slight differences in the localization of transfected neurons could explain such a difference: GABAergic cells in a more dorsal position within the LH and may be overflowing in the ZI could preferentially innervate the TRN. However an extensive description of the transfected area is missing to support this hypothesis or even formulate an alternative one. Despite these anatomical considerations, CNO-mediated activation of hM3DGq-expressing GABAergic neurons in the LH results in sustained waking, whereas the inhibition of the same neurons increases SWS amounts. The wake-facilitating effects of the stimulation of these GABAergic neurons could be mediated through their dense innervation of the POA and in particular the VLPO. However, previous work described that VGAT-expressing neurons in the LH fire maximally during SWS or REM sleep (Hassani et al. 2010) or increase their firing transiently after the transition from SWS to wake (Herrera et al. 2015). Moreover, a partially distinct population of GABAergic cells expressing GAD65 (GAD2) were found to be involved in stress-induced increase in locomotion downstream of Hcrt/Orx neurons (Kosse et al. 2017). All these results question the cellular complexity and heterogeneity of GABAergic neurons in the LH that serves as a major hub in multiple physiological responses and in particular where numerous allosteric factors affecting sleep-wake architecture could be integrated (see for reviews Bonnavion et al. 2016; Herrera et al. 2017).

One of these allosteric modulations could arise from the BNST, which is a constituent of the extended amygdala and plays a key role in stress responses and anxiety (Lebow and Chen 2016). Indeed, GABAergic GAD67 (GAD1)-expressing neurons of the BNST send dense axonal projections to the LH as well as to other brain areas involved in waking control such as the TMN, the locus coeruleus (LC), the dorsal raphe nucleus (DRN), or the dorsal deep mesencephalic reticular nucleus (dDpMe) (Kodani et al. 2017). The acute activation of these neurons using optogenetic stimulation during SWS episodes or a more sustained activation using DREADD tools resulted in increased wakefulness associated with an increased activation of Hcrt/Orx neurons in the LH and noradrenergic neurons in the LC quantified through cFos immunodetection. Interestingly, the arousal effects induced

by DREADD-mediated activation of GABAergic BNST neurons was reversed by the administration of the dual Hcrt/Orx receptor antagonist DORA-22, whereas it was not the case for the acute optogenetic activation of the same neurons (Kodani et al. 2017). Thus, GABAergic BNST neurons could play a key role in the SWS-to-wake transition without recruiting Hcrt/Orx neurons, while their sustained activation would involve Hcrt/Orx neurons to maintain waking. Additionally, the axonal projection of GABAergic BNST neurons to the LH was previously found to strongly drive food consummatory behaviors (Jennings et al. 2013). Thus, BNST neurons could provide a powerful allosteric modulation of LH neurons and potent other brain structures to drive and maintain arousal required for food-related behavior completion.

GABAergic Control of Waking in the Ventral Medulla

Multiple studies over the years highlighted the importance of GABAergic/glycinergic (GABA/Gly) neurons in the ventral medulla in the control of muscle atonia associated with REM sleep potentially acting as inhibitory premotor neurons through their descending projections to the spinal cord (Fraigne et al. 2015).

Recently, one paper using DREADD probed the role in the control of wakefulness and REM sleep atonia of GABA/Gly neurons in the ventromedial medulla at different levels in the rostrocaudal extent of the medulla (Chen et al. 2017). Chen and colleagues studied in parallel two portions of the ventromedial medulla: the ventromedial medulla rostral to the inferior olive (pSOM) and the rostral ventromedial medulla (RMV). Anatomically, RMV comprises the rostral raphe magnus (RMg), the raphe interpositus (RIP) nucleus, and the medial portion of the rostral gigantocellular reticular nucleus (Gi), whereas pSOM comprises the caudal part of the RMg, a centromedial portion and pars alpha of the Gi (GiA), and the rostral raphe obscurus nucleus (ROb). Diphtheria toxin-mediated ablation of either pSOM neurons or RMV neurons does not produce significant alteration in the sleep-wake architecture. However, lesions of the pSOM area cause an increase in phasic brief muscle activity during REM sleep recorded in the dorsal neck muscles without affecting tonic atonia. This suggests a role of pSOM neurons in the repression of twitches-like events during REM sleep. DREADD-mediated activation of RMV neurons whatever their neurochemical identity in rats induces increase in waking at the expense of both SWS and REM sleep, whereas activation of pSOM neurons unexpectedly induces a suppression of REM sleep compensated by a non-significant increase in waking and associated with a non-significant decrease in SWS. No effect was observed on REM sleep atonia or twitches-like events during REM sleep. The effect of pSOM neurons activation in rats was replicated in mice by specifically targeting DREADD expression in GABA/Gly neurons in the pSOM area. The above results reinforce the critical contribution of some GABA/Gly neuronal subpopulations in the control of REM sleep atonia and wake entry. It also contributes to highlight the complexity of neuronal networks controlling either REM sleep or REM sleep atonia in the ventral medulla. Indeed one population of GABA/Gly neurons in the ventral medulla located mostly in the lateral paragigantocellular nucleus (LPGi) and in the most ventral portion of the caudal Gi was found to be important in the promotion of REM sleep through ascending projections to the

ventrolateral periaqueductal gray (Weber et al. 2015). Conversely the suppression of GABA/Gly neurotransmission in adjacent neurons in the GiA, GiV and RMg causes a loss of muscle atonia during REM sleep associated with increased twitches mimicking symptoms of REM sleep behavior disorder (RBD) (Valencia Garcia et al. 2018). The upstream neurons of these systems controlling REM sleep onset or REM sleep atonia would be the glutamatergic neurons of the sublaterodorsal nucleus (SLD), the main REM sleep-promoting center, whose loss-of-function impedes REM sleep and produces similar RBD-like phenotype (Valencia Garcia et al. 2017).

3.2 Glutamatergic Systems

Multiple glutamatergic (Glu) neuronal populations were identified to contribute to the regulation of vigilance states. In comparison with the GABAergic systems that roughly facilitate sleep occurrence, Glu neurons hold a more versatile position (Fig. 1).

3.2.1 Glutamatergic Neurons in the Diencephalon

A first group of Glu neurons whose role in sleep-wake control has been evaluated using DREADD is located in the supramammillary nucleus (SUM) (Pedersen et al. 2017). Located in the caudal hypothalamus, the SUM exerts strong modulatory effects on the hippocampal formation and temporal cortex, in particular in relation to the control of theta rhythms (Thinschmidt et al. 1995). These neurons seem to play important roles during wakefulness in the control of anxiety-related behaviors, physiological stress, and defensive actions (see for review Pan and McNaughton 2004). In relation with sleep-wake regulation, SUM neurons strongly express the immediate early gene cFos after a REM sleep hypersomnia following REM sleep deprivation using the platform-over-water method and seem to be important for the activation of the dentate gyrus (DG) associated with REM sleep hypersomnia and theta rhythm during REM sleep (Renouard et al. 2015). Importantly, the majority of DG-projecting SUM neurons might be able to release both Glu and GABA (Soussi et al. 2010; Billwiller et al. 2017).

Chemogenetic interrogation of the role of SUM neurons in sleep-wake control was performed by injecting AAVs carrying cre-dependent DREADD constructs in the posterior hypothalamus of *VGlut2-cre* mice (Pedersen et al. 2017). The resulting transfected area indeed covered the entirety of the SUM but also extended more caudally in some portions of the posterior hypothalamus and the lateral hypothalamus. CNO-induced stimulation of hM3DGq-transfected neurons results in sustained wakefulness lasting from 6 to 10 h. This effect is almost completely abolished when knocking down VGlut2 in hM3DGq-expressing neurons. The remaining wake-promoting effects could be mediated through GABA release from the same neurons as CNO-induced stimulation of VGAT-expressing neurons produces similar effects. However, the size of transfection sites and the atypical absence of sleep rebound following prolonged wakefulness might raise the question about the specificity of the effects observed. Moreover, activation of DREADD-transfected Glu neurons

increases theta rhythms, which is consistent with previous reports (see for review Pan and McNaughton 2004), and promotes gamma oscillations. The latter observation should be interpreted with caution as EEG gamma rhythms can be easily contaminated by muscle activity (Buzsáki and Schomburg 2015). Conversely, CNO-induced inhibition of Glu neurons in a large area covering the SUM only produces slight effects with a small increase in sleep amounts and number of sleep episodes (Pedersen et al. 2017). Spectral analysis also indicates a reduction in theta oscillations during wake and a slowdown of theta rhythm during REM sleep that are consistent with previous observations during wake or REM sleep after deletion of SUM neurons (Pan and McNaughton 2004; Renouard et al. 2015).

Based on the contributions of SUM neurons in anxiety-related behaviors, stress, and defensive responses, the nature of the evoked-awakening and arousal state resulting from SUM DREADD-driven excitation remains unclear, whether it could actually be associated with high stress levels and anxiety. As with other arousal systems such as the Hcrt/Orx neurons (Bonnavion et al. 2015) and LC noradrenergic cells (McCall et al. 2015), sustained activation of such system with either semi-chronic optogenetic photostimulation or DREADD-driven activation might engage stress responses. However, considering the extension of the viral diffusion here, some of these neurons could also correspond to numerous posterior hypothalamic neurons, distinct from His neurons, displaying either a wake-active or a wake/REM sleep-active activity profile (Takahashi et al. 2006). Further investigations examining the activity profile of these neurons with particular regard to theta rhythm modulation during active wake and also during REM sleep would help in identifying the role of SUM in sleep/wake regulation. Considering the diversity of SUM neurons, anatomical studies of their respective connectivity would also further precise their position in sleep-wake regulatory networks.

3.2.2 Glutamatergic Control of Sleep-Wake Cycle in the Pontine Tegmentum

Glutamatergic neurons of the pontine reticular formation have a determinant role in sleep-wake state control (see for review Watson et al. 2011). Alongside the reticular formation, more defined nuclei containing REM-sleep-active Glu cells such as the laterodorsal (LDT) and pedunculopontine (PPT) tegmental nuclei and the sublaterodorsal nucleus (SLD) arose (see for review Brown et al. 2012). More recently, Hayashi and colleagues identified a subpopulation of neurons that derive from the cerebellar rhombic lip (a part of the developing hindbrain), transiently expressing the transcription factor Atoh1 at embryonic day 10.5 (E10.5), and that migrate to the pontine tegmentum (Hayashi et al. 2015). Atoh1 cells are either cholinergic or Glu neurons. A specific targeting of Atoh1 neurons was achieved by crossing *Atoh1-CreER*^{T2} mice with mice carrying a Cre-dependent tetracycline transactivator (tTA) transgene (*CAG-LSL-tTA*). Cre recombination and tTA expression in Atoh1 neurons were obtained with tamoxifen administration at E10.5 (Hayashi et al. 2015). AAVs carrying a tTA-dependent hM3DGq transgene were subsequently infused in either the medial or the lateral portion of the Atoh1 cell field in adult offspring. Thus, medial Atoh1 cells are localized in the ventral LDT and

medial parabrachial nucleus (PBm), and lateral Atoh1 cells are localized in the lateral portion of the PB (PBl). At the physiological level, in an extensively controlled experimental design, the authors observed that CNO-induced stimulation of medial or lateral Atoh1 cells increases SWS amounts and inhibits REM sleep or enhances wakefulness at the expense of both SWS and REM sleep, respectively (Hayashi et al. 2015). These effects seem to be mediated by Glu Atoh1 cells as the exact same effects were reproduced in transgenic mice obtained by crossing *Atoh-CreER*^{T2} mice with either *CaMkIIa-LSL-tTA* or *VGlut2-LSL-tTA* mice, in which tTA expression only occurs in Glu cells from the Atoh1 lineage (Hayashi et al. 2015). This nicely designed study led to the identification of two subpopulations of Glu cells that derive from the same developmental lineage and contribute to two distinct and yet complementary aspects of sleep regulation: lateral Atoh1 neurons that favor wake at the expense of sleep and medial Atoh1 neurons that facilitate SWS at the expense of REM sleep. These two subpopulations would act as gatekeepers of sleep and REM sleep, respectively.

Furthermore, Hayashi and colleagues dissected the neuronal pathway responsible for the REM sleep inhibiting effect of Glu medial Atoh1 neurons. They observed that these neurons densely innervate the dorsal deep mesencephalic reticular nucleus (dDpMe), which, alongside ventrolateral periaqueductal gray (vlPAG) GABAergic neurons, project and inhibit SLD neurons to suppress REM sleep (Fort et al. 2009; Sapin et al. 2009). CNO-mediated excitation of GABAergic dDpMe neurons expressing hM3DGq recapitulates the phenotype observed after the stimulation of medial Atoh1 cells, i.e., a decrease in REM sleep and an increase in SWS (Hayashi et al. 2015). Reciprocally, the inhibition of the same neurons expressing hM4DGi facilitates REM sleep onset. The latter effect is thus hypothesized to be mediated by a disinhibition of SLD REM sleep-promoting neurons.

Interestingly, wake-promoting lateral Atoh1 neurons partially overlap with neurons expressing Glu calcitonin gene-related peptide (CGRP) located in the external part of the lateral PB (PBel) (Bester et al. 1997; Yokota et al. 2015) As for Atoh1 lateral cells, the activation of CGRP positive cells using cre-dependent expression of hM3DGq in *CGRP-CreER* mice results in increasing wake at the expense of both SWS and REM sleep (Kaur et al. 2017). Moreover, optogenetic acute activation of these neurons during sleep reduces latency from sleep to wakefulness. PBel CGRP neurons, which are strongly activated in the context of hypercapnia (Yokota et al. 2015), appear to mediate their awakening signal through multiple excitatory projections into the central nucleus of the amygdala, the BF, and the LH. Altogether, these findings can be quite relevant to study circuit dysfunctions associated with obstructive sleep apnea or developmental defects that might occur in sudden infant death syndrome (SIDS) involving reduced CO_2 responses.

Finally, DREADD-mediated stimulation of a larger proportion of the PB complex was found to produce sustained wakefulness for more than 10 h (Qiu et al. 2016a). Similar wake-promoting effects are partially recapitulated by using a retrograde-driven expression of hM3DGq receptors in PB neurons projecting to the POA and the BF or projecting to the lateral and posterior hypothalamus. Conversely, no effect

is observed by stimulating PB neurons projecting to the midline and intralaminar thalamus (Qiu et al. 2016a). Strikingly, this sustained wakefulness is not followed by sleep rebound even after 4 days of daily CNO administration. In addition, cFos staining after this subchronic CNO treatment reveals a strong level of activation in many arousal-related centers including the LC, the TMN, the cholinergic BF, and the LH Hcrt/Orx neurons and in sleep-promoting areas, namely, the VLPO or the PZ. Indeed, the PB was found to be the main output structure of the sleep-promoting PZ (Anaclet et al. 2012, 2014) (Sect. 3.1.2). This cFos pattern that resembles both wake and sleep states is quite confusing and leaves open the question of the physiological relevance of this model and the multifaceted role of the PB complex in sleep/wake regulations.

3.3 Cholinergic Systems

Another major group of systems constitutive of the ascending arousal systems include cholinergic (ACh) neurons located in the pedunculopontine (PPT) and laterodorsal (LDT) tegmentum nuclei and in the basal forebrain (BF) (see for review Fort et al. 2009). These neurons are mostly active during both waking and REM sleep in association with cortical activation characterizing these two vigilance states. DREADD approaches brought significant revision on the role of ACh from both the BF and brainstem by highlighting the cell diversity including GABAergic and glutamatergic neurons in these brain areas and their respective contributions (Fig. 1).

3.3.1 Pontine Cholinergic Systems

Localized within LDT and PPT nuclei, ACh neurons in the mesencephalic tegmentum have long been associated with cortical desynchronization during waking and REM sleep (Steriade 2004; Boucetta et al. 2014) through projections to the forebrain and to the brainstem reticular formation. Moreover, acute optogenetic stimulation of ACh cells in either the PPT or the LDT during SWS leads to REM sleep onset within a minute (Van Dort et al. 2015). However ACh neurons are also intermingled with GABAergic and Glu cells that could contribute to sleep architecture, as they are maximally active during waking or REM sleep or both (Boucetta et al. 2014). Recently, one study tried to dissect the respective contributions of ACh, GABAergic, and Glu cells in the PPT using DREADD-mediated cell-specific modulation of their activity (Kroeger et al. 2017). CNO-mediated activation of VGlut2-expressing PPT cells results in increased waking for at least 4 h at the expense of SWS and a complete inhibition of REM sleep. This CNO-induced waking is mostly composed of quiet wake in the nest and is associated with increased levels of anxiety. Conversely, inhibition of the same neuronal group increases SWS, reduces waking, and lets REM sleep amounts unaffected (Kroeger et al. 2017). CNO-mediated excitation of hM3DGq-expressing ACh neurons of the PPT increases light SWS at the expense of deep SWS without affecting either wake or REM sleep amounts in sharp contrast with results harvested using optogenetic stimulation of the same neurons (Van Dort et al. 2015). This discrepancy could be due to a low transfection rate (about 20%) of

cholinergic cells obtained by Kroeger and colleagues. However, in an attempt to reconcile these findings, CNO-induced stimulation of PPT ACh cells could primarily facilitate the appearance of a transition sleep state between SWS and REM sleep, thus gating REM sleep onset (Mandile et al. 1996; Vescia et al. 1996). Eventually, the activation of local GABAergic neurons only mildly reduces REM sleep despite the fact that identified GABAergic cells in the PPT discharge maximally during REM sleep only or during both wake and REM sleep across the spontaneous sleep-wake cycle (Boucetta et al. 2014). Generally, as clearly stated by the authors, the overall results should be interpreted with caution as targeting precisely and specifically a small brain area in mice using viral vectors is challenging, especially in the pontine tegmentum that concentrate multiple nuclei antagonistically regulating the sleep-wake architecture.

3.3.2 Basal Forebrain Control of Sleep-Wake Cycle

Numerous studies pointed out a key role of BF, and especially BF ACh neurons, in the regulation of the sleep-wake cycle in particular in relation to cortical activation during waking or REM sleep (see for review Fort et al. 2009). However it remains unclear which BF neuronal subpopulations contribute to a given vigilance state and how they interact with each other and affect BF projection areas. In addition to ACh neurons, BF contains intermingled Glu and GABAergic cells that are also likely to play pivotal roles in sleep-wake regulation. Over the last few years, multiple studies tried to decipher the relative contributions of these subpopulations using either optogenetics or DREADDs.

In vivo recordings of channelrhodopsin-2-tagged neurons in the magnocellular preoptic area (MCPO) and the nucleus of the horizontal limb of the diagonal band (HDB) confirmed that ACh and Glu BF neurons display higher firing rates during wakefulness and REM sleep than during SWS (Xu et al. 2015). Interestingly, when considering GABAergic cells, two subpopulations with opposite modulation during spontaneous sleep-wake cycles emerged: parvalbumin (PV)-expressing are more active during wake and REM sleep, whereas some somatostatin (SOM)-containing cells would discharge maximally during SWS (Xu et al. 2015). The subsequent optogenetic activation of these subpopulations confirmed the above correlative characterization: stimulation of BF ACh neurons results in increased transition probability from SWS-to-wake or to a lesser extent from SWS-to-REM sleep; stimulation of either Glu or PV-expressing neurons facilitates wakefulness at the expense of SWS; and stimulation of SOM-containing neurons increases SWS at the expense of wakefulness (Han et al. 2014; Xu et al. 2015).

The same deconstruction of BF subpopulation contributions to sleep physiology was also conducted through DREADD-mediated modulation of their activity. CNO-mediated stimulation of ACh neurons in the HDB, the MCPO, the substantia innominata (SI), and the ventral pallidum (VP) does not modify the sleep-wake architecture but decreases slow-wave activity during SWS (Anaclet et al. 2015). Conversely, the same approach restricted to ACh cells in the HDB, the MCPO, and the SI results in increased wakefulness associated with decreased slow-wave activity during SWS (Chen et al. 2016). Moreover, the CNO-driven inhibition of the same

neurons produced the exact opposite phenotype (Chen et al. 2016). DREADD-mediated stimulation of Glu cells in the HDB, MCPO, SI, and VP is inefficient on sleep architecture and mildly decreases delta power during SWS (Anaclet et al. 2015). In contrast, CNO-induced stimulation of GABAergic neurons in the whole BF increases wake during more than 6 h at the expense of both SWS and REM sleep associated with an increase in EEG spectral power in the alpha and gamma bands (Anaclet et al. 2015). The latter effects on gamma rhythms would preferentially involve BF PV-expressing neurons (Kim et al. 2015).

The above studies provided additional clues into the respective contributions of various BF cellular subpopulations into the regulation of sleep-wake architecture as well as their contributions to brain oscillatory activities associated with either wake or SWS. However inconsistent observations were reported and could be dependent on the extent of the brain area considered: some restricted their targeting strategy to the HDB/MCPO region (Xu et al. 2015; Chen et al. 2016), whereas other included the VP (Han et al. 2014; Anaclet et al. 2015). The anatomical delineation of the sleep/wake-related BF may be a critical point, and the question to incorporate a given brain region into the "classical" ascending activating systems should be further addressed and discussed. Indeed, the VP is part of the basal ganglia, and thus its activation can directly influence motivation and locomotor behaviors through the limbic loop (see for review Root et al. 2015). An additional recruitment of VP could explain the long-lasting wake-promoting effect of GABAergic neuron stimulation obtained by Anaclet and colleagues (2015) in comparison with a more localized optogenetic stimulation of PV neurons in the HBD/MCPO (Anaclet et al. 2015; Xu et al. 2015). As a consequence, redefining the respective contributions of the different subregions (i.e., HDB, MCPO, SI, VP) would be a critical point to address. As the projection patterns of VP neurons differ from MCPO/HDB neurons, associating genetic tagging to retrograde mapping strategies would be of great use, in particular in mice in which targeting a small region with viral delivery is highly challenging.

3.4 Monoaminergic Systems

3.4.1 Serotonin

Serotonergic (5-HT) neurons located in the raphe nuclei belong to the ascending arousal system that triggers cortical activation during wake. Indeed, most of 5-HT neurons discharge maximally during wake, decrease their firing rate during SWS, and are silent during REM sleep (McGinty and Harper 1976; Rasmussen et al. 1984; Sakai 2011). As a result, extracellular 5-HT levels are higher during wake than during sleep both in the raphe nuclei and in brain areas targeted by 5-HT neurons (Portas et al. 2000). Nevertheless, 5-HT can also contribute to increase sleep propensity, as first observations showed that either lesions of 5-HT neurons of the raphe system or inhibition of 5-HT synthesis induce a severe insomnia which could be reversed by restoring 5-HT synthesis (Jouvet 1999). Importantly, enhancing 5-HT tone by systemic administration of selective serotonin reuptake inhibitors (SSRIs)

has been repeatedly reported to inhibit REM sleep across species (Slater et al. 1978; Sommerfelt and Ursin 1987; Maudhuit et al. 1994; Monaca et al. 2003). Thus, upon the different approaches used, while 5-HT was first believed to be a neuromodulator of sleep, it is now further thought to promote cortical activation while impeding REM sleep onset during wake. Still its role in sleep-wake regulation remains very unclear, and these differential effects involve complex modalities of action and multiple receptors in various brain structures (Ursin 2002) challenging investigations over 5-HT system.

Despite the development of novel techniques including DREADD and optogenetics, the reassessment of 5-HT role in sleep has been underexplored. Interestingly, the very first in vivo validation using inhibitory DREADD approach was implemented to the entire 5-HT system (Ray et al. 2011). Conditional intersectional genetics were used to switch on expression of the hM4DGi transgene in virtually all serotonergic neurons of *Slc6a4* (5-HT transporter)-*cre* or *Pet1-Flpe* mice (Ray et al. 2011). Acute inhibition of the entire 5-HT system, following CNO i.p. injection (10 mg/kg), induces a dramatic drop in body temperature and disrupts chemoreflex in response to CO_2 elevation. This first in vivo approach validating DREADD inhibitory control applied a unique dose of CNO that is now considered extremely high. However this study acutely shutting down the 5-HT system was aimed to model homeostatic dysfunctions involving significant 5-HT abnormalities occurring in fatal or life-threatening disorders such as in the sudden infant death syndrome (SIDS).

More recently, interesting findings using DREADD and optogenetic approaches have shown that 5-HT neurons of the dorsal raphe (DR) inhibit the pathological intrusion of REM sleep into wake in a mouse model of narcolepsy, highlighting a pharmacogenetic approach for the amelioration of narcolepsy (Hasegawa et al. 2014, 2017).

Narcolepsy, which is characterized by excessive daytime sleepiness and cataplexy, is associated with the loss of Hcrt/Orx neurons (Dauvilliers et al. 2003). Mice lacking Hcrt peptides, Hcrt neurons, or Hcrt receptors recapitulate human narcolepsy phenotypes (Taheri et al. 2002; Hasegawa et al. 2014). In particular, there are two features that these models share with human pathology: inability to maintain consolidated wakefulness and abrupt behavioral arrests with muscle atonia resembling cataplexy. In their first study, Hasegawa and colleagues investigated the primary wake-active target of Hcrt neurons that could mediate the suppression of narcoleptic symptoms and showed that 5-HT cells of the dorsal raphe (DR) and noradrenergic (NA) cells of the LC have differential roles (Hasegawa et al. 2014). First, in narcoleptic mice devoid of Hcrt/Orx receptors, the restoration of Hcrt2-R in DR 5-HT cells prevents cataplexy-like episodes (CLEs) and restores REM sleep amounts, whereas wake fragmentation persists. In contrast, restoration of Hcrt1-R in LC NA neurons corrects wake fragmentation by increasing mean duration of wake episodes and decreasing the number of wake episodes, without affecting CLEs or REM sleep hypersomnia (Hasegawa et al. 2014). Next, to further examine whether activation of 5-HT DR neurons and NA LC neurons could rescue narcoleptic symptoms, Hasegawa and colleagues implemented DREADD approach in another

model of narcoleptic mice in which Hcrt/Orx neurons are specifically ablated (*orexin/ataxin-3* mice). To combine this approach in their model, they used viral vectors stereotaxically injected into the DR or LC carrying DREADD transgene expression under the control of promoters specific to 5-HT or NA neurons (Pet-1 or PRSx8, respectively) of Hcrt/Orx-ablated mice. Importantly, DREADD activation of DR 5-HT neurons and LC NA neurons reverses the occurrence of CLEs and wake fragmentation, respectively, as seen when restoring Hcrt/Orx receptor expression (Hasegawa et al. 2014). This study allowed the identification of two pathways differentially regulating narcoleptic symptoms that highlights a critical role of DR 5-HT neurons in regulating REM sleep excess and CLE prevention, while LC NA system would rather control wakefulness maintenance.

In their latest work, Hasegawa and colleagues further investigated how DR 5-HT neurons mediate the suppression of CLEs (Hasegawa et al. 2017). By reproducing optogenetically the anti-cataplectic effects of DR 5-HT activation in orexin/ataxin-3 mouse model, they showed that stimulation of DR 5-HT fibers directly into the amygdala was sufficient to suppress the occurrence of CLEs, but did not correct REM sleep hypersomnia or wake fragmentation (Hasegawa et al. 2017). The photostimulation of 5-HT terminals also induced decreased expression of cFos in the amygdala in comparison with the non-stimulated condition in narcoleptic mice, suggesting that 5-HT may inhibit the amygdala to suppress CLEs. To further confirm the importance of the inhibition of amygdala activity to prevent CLEs, the authors employed direct DREADD inhibition of amygdala neurons by locally injecting a pair of AAV vectors: AAV-EF1α-DIO-hM4Di-mCherry and AAV-SynI-iCre in orexin/ataxin-3 narcoleptic mice. CNO treatment substantially reduces CLEs in comparison with saline treatment (Hasegawa et al. 2017). Consistently with 5-HT effects, DREADD inhibition of the amygdala does not improve wake fragmentation. In a complementary DREADD approach in these mice, Hasegawa and colleagues expressed the excitatory hM3DGq DREADD transgene in the amygdala and observed that CNO-driven activation of the amygdala significantly increases the occurrence of CLEs without affecting wake fragmentation (Hasegawa et al. 2017). Thus, chemogenetic manipulations of amygdala activity modulate specific symptoms of narcolepsy resembling cataplexy in a bidirectional manner in orexin/ataxin-3 narcoleptic mice. Finally, the authors designed a nice combination of molecular tools with optogenetics to demonstrate the specific involvement of DR 5-HT-amygdala pathway in the suppression of Hcrt-dependent CLEs showing that the anti-cataplectic effects of restoring Hcrt2-R in DR 5-HT neurons were blocked with the optogenetic inhibition of the amygdala in narcoleptic mice lacking both Hcrt receptors (Hasegawa et al. 2017).

In narcoleptic patients as well as in narcoleptic dogs and mice, cataplexy is most often triggered by positive emotions (Dauvilliers et al. 2003; Taheri et al. 2002). The DREADD results obtained in this study further link the amygdala, which is important for emotional processing, to the complex physiopathology of narcolepsy. It also suggested that the control of 5-HT on the excess of REM sleep and cataplexy in narcoleptic mice involves distinct pathways and mechanisms, as DREADD activation of DR 5-HT improved both excessive REM sleep duration and CLEs in

narcoleptic mice but optogenetic stimulation of 5-HT fibers in the amygdala or direct DREADD activation of amygdala neurons only modulated CLEs. It still poses certain questions on the nature of the anti-cataplectic actions of 5-HT in the amygdala whether it might be associated to fear or reward processing. The amygdala is a complex structure characterized by a central nucleus and a basolateral complex with diverse functions, responding to both aversive (fear) and positive (reward) signals (see for review Janak and Tye 2015). It remains discussed whether 5-HT neurotransmission in the amygdala participates to the acquisition of cued conditioned fear (see for review Bauer 2015), but it still questions whether 5-HT-mediated suppression of CLEs in the amygdala could be produced by fear. Further investigations on amygdala subnuclei and pathways involved in this model should help in shedding light on the circuit and mechanisms underlying these effects and better identify the role of 5-HT in narcolepsy.

3.4.2 Noradrenaline

The main source of noradrenaline (NA) in the central nervous system is supplied by the locus coeruleus (LC) through diverse and widespread efferent projections. Similarly to histamine and serotonergic systems, LC NA neurons are wake-active with the particularity that they start to fire before the onset of wake, suggesting a role in wake induction (Aston-Jones and Bloom 1981; Takahashi et al. 2010). During wakefulness, LC NA neurons show distinct tonic and phasic patterns of activity: tonic discharge rate is positively correlated to states of arousal with notable increase in firing when the animals encounter unexpected novelty or perform operant-discrimination tasks, while phasic activations characterized by brief excitatory component followed by a longer duration of inhibition are associated with discrete sensory stimuli (see for reviews Aston-Jones et al. 1999; Sara 2009). Other studies have also associated tonic and phasic activation of LC neurons in response to stressors (see for review Aston-Jones et al. 1999). LC NA neurons cease firing just before the onset of SWS and remain silent during both SWS and REM sleep (Takahashi et al. 2010). The robust wake-promoting actions of LC NA neurons were further evidenced with optogenetics (Carter et al. 2010). Stimulating LC NA neurons optogenetically at 5 Hz induces arousal and can trigger wakefulness from NREM sleep (Carter et al. 2010). However in contrast with histamine system (see Sect. 3.4.4) (Fujita et al. 2017), acute inhibition of LC NA neurons does not immediately alter the behavioral status of the mice by switching from wake to sleep (Carter et al. 2010). Indeed, only a 1-h-long semi-chronic inhibition progressively drives an increase of sleep amounts at the expense of wakefulness (Carter et al. 2010). To date, in our knowledge, pharmacogenetic manipulation of LC NA neurons in sleep studies has been used in one study in rats investigating the causal relationship between LC NA activity and general anesthetic state (Vazey and Aston-Jones 2014). By generating a vector allowing hM3DGq transgene expression in LC NA neurons using the synthetic dopamine-β-hydroxylase (DBH) promoter PRSx8 in rats, Vazey and Aston-Jones tested the role of LC NA activation during general anesthesia and in the emergence from it. To do so, CNO was first administered locally and unilaterally into the LC while monitoring the cortical EEG of deeply

anesthetized rats under continuous isoflurane (2%) exposure. The authors observed that CNO microinjection in LC-hM3DGq rats leads to cortical EEG activation with a significant decrease in delta band power and increase in theta band power, in comparison with either vehicle microinjections or LC-mCherry control rats (Vazey and Aston-Jones 2014). DREADD-driven activation of LC NA neurons during anesthesia also induces an increase in total EEG power consistent with less burst suppression in cortical EEG, which is a measure of anesthetic depth, indicating a transitioning state away from deep anesthesia. Consistently, systemic CNO activation of LC NA neurons accelerates emergence from isoflurane anesthesia measured by rapid return of righting reflex after discontinuation of isoflurane, and that was shown to be mediated through both β and α1 adrenergic receptors (Vazey and Aston-Jones 2014). These findings showed that selective activation of LC NA neurons is sufficient and powerful enough to overcome the general anesthetic effects of artificially strong GABAergic inhibition, and trigger arousal. LC NA neurons specific implication and efficiency to promote arousal has been also highlighted in mice models of narcolepsy in which DREADD activation of LC NA neurons corrects wake fragmentation associated with narcolepsy (Hasegawa et al. 2014) (see Sect. 3.4.1).

Application of DREADD technique to LC NA neurons has been also used to stimulate degenerating neurons and test beneficial effects of increasing brain NA levels on cognitive impairments in a mouse model of Down syndrome (Rorabaugh et al. 2017) and in a rat model of Alzheimer's disease (Fortress et al. 2015). In both models DREADD stimulation of LC NA neurons results in cognitive improvements. Interestingly, another recent study using DREADD control established a causal relationship between LC NA neurons activity and stress-induced anxiety showing that hM4DGi-driven inhibition of LC NA neurons during stress prevents subsequent anxiety-like behavior, and in contrast, increased tonic hM3DGq-driven activation of LC NA neurons is sufficient to promote anxiety-like and aversive behavior (McCall et al. 2015). Interestingly, the authors found that the effects of LC stimulation on acute anxiogenic responses are blocked by the β-adrenoreceptor antagonist, whereas the aversive effects require α1-adrenoceptor activity, suggesting that acute stress-induced anxiety and aversive behaviors are driven by distinct circuits. Indeed, NA has simultaneously excitatory and inhibitory signaling through α1/β receptors and α2 receptors, respectively. While NA system produces arousal and deepens cognition, or takes part in stress-induced responses, selective pharmacological activation of α2 receptors produces deep SWS. This class of α2 receptor selective agonists belongs to prominent sedative drugs used for long-term sedation in hospital intensive care units (dexmedetomidine) or in veterinary clinics to sedate animals (xylazine) (see for review Yu et al. 2018). Thus, apart from arousal-promoting functions covering various physiological and cognitive processes or emotional responding, NA can also have sleep-promoting contributions (Grivel et al. 2005). All together these studies highlight the diversity of functions of NA that should be further addressed in light of the complex input/output organization of the LC (Schwarz et al. 2015) but should also integrate arousal-enhancing actions originating

from other groups of NA-releasing cells including brainstem A1 and A2 nuclei (Berridge 2008).

3.4.3 Dopamine

Among monoamines, dopamine (DA) seems to hold a notable position, being excluded from the "classical" ascending wake-promoting monoaminergic systems. This might be the consequence of the initial demonstration of an absence of change in neuronal activity of VTA DA cells across the natural sleep-wake cycle (Miller et al. 1983; Steinfels et al. 1983).

One recent paper using DREADD among other methods contributed to reintegrate DA neurons in sleep-wake regulating networks (Eban-Rothschild et al. 2016). Using fiber photometry on GCaMP-expressing DA neurons, the authors observed that, at a population level, DA neurons of the ventral tegmental area (VTA) are more active during wake and REM sleep than during SWS. Interestingly, the change in fluorescence is significantly higher during REM sleep bouts than waking bouts. This might be linked to the actual discharge pattern of VTA DA neurons across the natural sleep-wake cycle and the bursting pattern they display during REM sleep or during wakefulness in relation to appetitive or rewarding events (Dahan et al. 2007). This observation is also consistent with microdialysis measurements of DA in the NAc or the mPFC that revealed increased concentration of DA during wake and REM sleep compared to SWS (Léna et al. 2005). Using DREADD techniques, Eban-Rothschild and colleagues found that hM4DGi-driven inhibition of VTA DA neurons soon after the onset of the dark active photoperiod results in decreased wakefulness compensated by an increase in both SWS and REM sleep (Eban-Rothschild et al. 2016). These effects even persisted when animals are facing a salient stimulus such as palatable food, female mouse, or predator odor. Strikingly, the inhibition of VTA DA also facilitates the appearance of active nest-building behavior prior to fastened sleep onset only in the context of an absence of available nest. The authors suggested that these observations indicate that VTA DA neuron activity is necessary for preparatory sleep-related behaviors.

Conversely, optogenetic stimulation of VTA DA neurons during diurnal inactive period results in short-onset awakening (Eban-Rothschild et al. 2016), and the same effect was observed after DREADD hM3DGq activation of the whole VTA (Sun et al. 2017). The above optogenetic effect was replicated by stimulating DA terminals specifically in the NAc, in the DLS, and in the central nucleus of the amygdala, but not in the mPFC (Eban-Rothschild et al. 2016). Similarly, a deletion of SNc DA neurons in rats increases waking, whereas the optogenetic stimulation of DA terminals in the dorsal striatum inhibits wake in favor of SWS (Qiu et al. 2016b). One of the main targets of DA cells in the context of sleep-wake regulation would thus lie in the striatum. As a consequence, when VTA/SNc DA neurons are active, D_1-expressing dMSNs in the striatum would be activated, whereas D_2/A_{2A}-expressing iMSNs facilitating sleep onset (Oishi et al. 2017; Yuan et al. 2017) would be inhibited (Sect. 3.1.4). These results thus provide evidence for a role of midbrain DA neurons in facilitating waking maintenance, in particular in the context of aversive or rewarding stimuli requiring an alerting response.

Midbrain DA cells are also strongly active during REM sleep and display a characteristic bursting firing pattern (Dahan et al. 2007) associated with large DA release (Léna et al. 2005) whose function remains unresolved. One possibility would be an involvement of DA transmission in memory consolidation processes. Indeed, the activity of VTA DA neurons seems to be associated with theta rhythm (see for review Orzeł-Gryglewska et al. 2015) that is preeminent during REM sleep, and multiple evidences highlighted a causal role of REM sleep in episodic memory consolidation (Datta et al. 2004; Inostroza et al. 2013; Ravassard et al. 2016) with a particular focus on theta rhythm (Nishida et al. 2009; Popa et al. 2010; Boyce et al. 2016). Moreover theta rhythm during REM sleep could putatively contribute to implicit memory formation (Santos et al. 2008). Midbrain DA neurons could thus play a significant role in memory consolidation, especially with respect to the sequential hypothesis (Ambrosini and Giuditta 2001; Giuditta 2014; Sara 2017), which proposes that "irrelevant" information would be downgraded during SWS, whereas "relevant" information would be tagged maybe through cell ensemble reactivations and then, sequentially, integrated into preexisting memories during REM sleep, thanks to fast theta oscillations and the expression of transcription factors that both seem to be under the influence of DA signal (Orzeł-Gryglewska et al. 2015; Rioult-Pedotti et al. 2015; Wieland et al. 2015).

3.4.4 Histamine

Until recently, the brain histaminergic (His) system was critically understudied in comparison with other arousal monoaminergic circuits. The use of optogenetics, DREADD approaches, and genetic models revealed important findings on the His neurotransmission and function (Fig. 1).

His neurons of the hypothalamic tuberomammillary nucleus (TMN) display unique electrophysiological signatures characterized by a slow pacemaking activity (1–5 Hz) (Reiner and McGeer 1987) that is tightly coupled to behavioral arousal and is the most wake-specific firing pattern of any cell type identified in the brain to date (Vanni-Mercier et al. 2003; Takahashi et al. 2006; Sakai et al. 2010). His neurons start to fire after waking onset, increase firing during attentive waking, and cease their activity before sleep onset to remain silent during all stages of sleep (Vanni-Mercier et al. 2003; Takahashi et al. 2006; Sakai et al. 2010). These data suggested that His neurons would play a role in the maintenance of wakefulness and in adapting optimal levels of arousal necessary for cognitive processes (Lin et al. 2011b). It also suggested that the cessation of His activity may be necessary for sleep induction, which was recently evidenced by optogenetic approaches (Chung et al. 2017; Fujita et al. 2017). Genetic loss-of-function manipulations and pharmacological data provide a consistent picture because impairment to the His neurotransmission results in sleepiness and behavioral deficits (Lin 2000; Parmentier et al. 2002; Anaclet et al. 2009), whereas its enhancement promotes wakefulness and displays pro-cognitive effects (see for review Haas et al. 2008; Schwartz 2011).

Recently, novel insights on His control of arousal and synaptic mechanisms associated with His neurotransmission were achieved in a complete study combining DREADD technique, optogenetics, and genetic models (Yu et al. 2015). First, Yu and colleagues observed that a sustained activation of the His system, using conditional Cre-mediated expression of hM3DGq in histidine decarboxylase (HDC)-expressing neurons (*HDC-Cre*), induces a strong behavioral change assessed by hyperlocomotion in an open field (Yu et al. 2015). The CNO dose used in this study was quite high (5 mg/kg), and effects were compared with saline i.p. injections. In light of the recent studies questioning CNO endogenous effects, further controls should be examined in future studies. However, if there were any endogenous effects of CNO through clozapine conversion, we could expect opposite behavioral responses as shown by Gomez and colleagues in a similar open field test (Gomez et al. 2017). The main question of this approach was to test the ability of the His system to evoke excitatory behavioral responses with a large activation of the His neurotransmission. As a result, DREADD activation of His neurons under high dose of CNO leads to long-lasting hyperactivity (Yu et al. 2015). Unfortunately, effects on sleep-wake cycle and EEG remain unknown.

Most importantly, by questioning the functional role of the presence of GABA in the vast majority of His neurons (Takeda et al. 1984; Senba et al. 1985; Trottier et al. 2002), Yu and colleagues showed that most His neurons use VGAT to release GABA and highlighted the importance of GABA co-release in controlling behavioral arousal (Yu et al. 2015). They showed that conditional knockdown and knockout mice with disrupted GABA function in His neurons are hyperactive, exhibiting hyperlocomotion and decreased amounts of sleep confined to the night in comparison with control mice (Yu et al. 2015). Using optogenetic stimulation of HDC-ChR2 fibers, they further demonstrated ex vivo that GABA is being directly released from His fibers in the neocortex and striatum. Interestingly in the VLPO, His axons do not release GABA when stimulated optogenetically but instead activate GABA interneurons by His release to suppress the activity of long-range projecting sleep-promoting neurons (Williams et al. 2014). Apart from technical differences, Yu and colleagues suggest that these contrasting results with neocortical neurons might be due to a subpopulation of His neurons projecting to the VLPO devoid of GABA (about ~20%), which is intriguing to test by further histological and tracing investigations considering the growing evidences of diversity among His neurons (Blandina et al. 2012; Fujita et al. 2017). Moreover, the striatum is also composed of a diversity of cells including two types of MSNs and various interneurons (Durieux et al. 2011). Thus, additional recordings in the distinct striatal neuronal populations should be conducted to precisely define the nature and mechanisms of His actions in the BG circuitry (Bolam and Ellender 2016).

The study led by Yu and colleagues emphasized a major phenomenon observed in other monoaminergic systems as subsets of 5-HT and DA neurons also have the ability to release Glu or GABA fast neurotransmitters through different vesicular transporters (see for review Lőrincz and Adamantidis 2017). As a consequence, it highlights the need to dissect in vivo effects with a circuit-level approach combining various techniques monitoring neuronal activity and/or release, notably when using DREADD or optogenetic approaches. Even if neurochemical specificity can be

achieved through genetic targeting, certain effects attributed to monoamine release can be in fact mediated or counteracted by fast neurotransmission. Why His-GABA neurons use contradictory signals remains an open question that is well-discussed by Yu and colleagues proposing that it could either serve to stop networks getting too excited by an overactive His system, which may have deleterious consequences on sleep and mental health as seen in their models. Conversely, it could actually better shape cognitive responses together with His, notably at the level of the cortex in adjusting the balance between synaptic excitation and inhibition to enhance processing (see discussion Yu et al. 2015).

Finally, the His system has emerged as an attractive therapeutic target for the treatment of neuropsychiatric and neurodegenerative diseases. His regulates its own release through autoinhibitory H3 receptors (H3R) (Lin et al. 2011a). Selective H3R blockers enhance both the firing rate of His neurons and His release (Lin et al. 2011a). Interestingly, administration of H3R blockers dramatically enhances the high-frequency component of neocortical EEG rhythms in cats, mice, and humans and improves attention and facilitates learning in rodent models (see for review Schwartz 2011), lending support to the role of His in higher cognitive processes. Selective blockers are now gaining entrance in clinical trials showing tremendous promise as a therapeutic intervention in schizophrenia, dementias, sleep disorders, and other conditions for its wake-promoting and pro-cognitive effects (Schwartz 2011). Thus, it is timely to further our understanding on the role and actions of TMN His neurons and His-GABA neurons in arousal and cognition.

3.5 Peptidergic Systems

3.5.1 Hypocretin/Orexin

Localized in the lateral hypothalamus, hypocretin/orexin (Hcrt/Orx)-secreting neurons are key contributors to wakefulness stabilization (Bonnavion and De Lecea 2010), and dysfunction of the Hcrt/Orx system is linked to narcolepsy (see Sects. 3.4.1 and 3.4.2). Pharmacosynthetic modulation of Hcrt/Orx neurons activity revealed that the CNO-elicited activation of hM3Dq-expressing Hcrt/Orx neurons increases wake at the expense of both SWS and REM sleep, whereas their inhibition facilitates SWS and decreases wake and REM sleep amounts (Sasaki et al. 2011). However, a more detailed description of the sleep-wake architecture after CNO/DREADD-driven control is still missing as acute optogenetic activation of Hcrt/Orx neurons during SWS produces Hcrt peptide-dependent awakening within a minute, whereas acute inhibition of the same neurons during wake accelerates SWS onset (Adamantidis et al. 2007; Tsunematsu et al. 2011). Hcrt/Orx neurons seem of particular importance for wake consolidation and proper control of sleep-wake dynamics without strongly affecting daily wake amounts (Hara et al. 2001; Diniz Behn et al. 2008, 2010; Kantor et al. 2009; De Lecea 2012). During wakefulness, Hcrt/Orx cells are not constantly firing and become stimulated in association with signals that require increased wakefulness (Yamanaka et al. 2003; Mileykovskiy et al. 2005; Williams et al. 2007; Bonnavion et al. 2015; González et al. 2016).

Altogether these findings reinforce the unique position of Hcrt/Orx neurons among wake-active systems that may thus be regarded as computational comparators generating an error signal to recalibrate arousal levels in comparison to the difference between the actual state and required arousal (Kosse and Burdakov 2014).

3.5.2 Melanin-Concentrating Hormone

Neurons expressing melanin-concentrating hormone (MCH) are a molecularly defined neuronal subpopulation largely distributed throughout the extent of the LH and ZI. Several experiments pointed out a pivotal contribution of MCH-secreting neurons in the control of sleep and in particular REM sleep. Indeed both optogenetic (Jego et al. 2013; Konadhode et al. 2013; Tsunematsu et al. 2014) and DREADD-mediated stimulations (Vetrivelan et al. 2016; Varin et al. 2018) of MCH neurons unfailingly result in facilitated REM sleep onset and increased REM sleep amounts. However, inconsistent and puzzling results on SWS were harvested by these studies, probably due to heterogeneous experimental and technical procedures. Indeed, the semi-chronic optogenetic stimulation of MCH neurons during 24 h extends SWS only during the nocturnal active phase by increasing the number of SWS bouts (Konadhode et al. 2013). Their acute stimulation at the onset of a given SWS episode lets the duration of the ongoing episode unaffected (Jego et al. 2013), whereas SWS amounts and the mean duration of SWS episodes are decreased upon continuous optogenetic stimulation of MCH neurons for 3 h during the light inactive photoperiod (Tsunematsu et al. 2014). In an attempt to solve these discrepancies, two groups recently examined the effects on sleep of the DREADD-mediated modulation of MCH neuron activity (Vetrivelan et al. 2016; Varin et al. 2018). The first study (Vetrivelan et al. 2016) analyzed the effects on sleep of CNO-induced activation of hM3DGq-expressing MCH neurons and confirmed previous evidences indicating that the stimulation of MCH neurons favors REM sleep onset and consolidation. The second one (Varin et al. 2018) combined the utilization of both hM3DGq and hM4DGi DREADD effectors. Varin and colleagues consistently confirmed the REM sleep-facilitating effect of MCH neuron activation and additionally observed that CNO-mediated stimulation of MCH neurons results in decreased SWS amounts, whereas the inhibition of MCH neurons resulted in the opposite phenotype. These effects were accompanied with modifications in SWS delta rhythmic activities suggesting that MCH neurons could participate in increasing SWS depth (Varin et al. 2018). Thanks to their observations using both excitatory and inhibitory DREADD tools and a multiple CNO doses design, the authors consequently proposed a novel formulation of the role of MCH neurons in sleep physiology, that is, MCH neurons would be involved in basic mechanisms occurring primarily during SWS aimed at deepening SWS to pave the way for SWS-to-REM sleep transition to occur.

4 Conclusion and Perspectives

Following the path opened by optogenetics more than a decade ago, the development and use of pharmacosynthetic tools quickly made their contribution to the discovery and the expansion of our understanding of critical brain structures and neuronal subpopulation involved in a wide variety of behaviors and in particular in the control of vigilance state architecture.

We now end up with a large – but probably not exhaustive – set of brain structures that are key modulators or serve executive functions in the control of the sleep-wake cycle (Fig. 1). This leads to a complex network of deeply interconnected and heterogeneous neuronal subpopulations (Fig. 2). Deciphering this complexity and unscrambling the hierarchical organization of the sleep-wake regulating network constitute a challenging task that sleep scientists are now facing. Indeed this challenge is particularly prominent when attempting to describe and quantify the series of activations and inhibitions of different brain structures and neuronal subpopulations occurring cooperatively and concurring to sharp and complete transitions between vigilance states.

In front of this demanding task, multiple novel tools offer new possibilities to move a step forward in our understanding of sleep physiology. Recent advances in in vivo electrophysiology provide opportunities to record multiple cellular subpopulations that can additionally be identified by optogenetic tagging at the same time in different brain regions in order to draw a finer picture of neuronal dynamics during vigilance states and transitions between states (Vyazovskiy et al. 2011; Herrera et al. 2015; Weber et al. 2015; Xu et al. 2015).

The hierarchical organization of sleep-wake systems could be probed by multiplexing various genetically encoded actuators or sensors. For instance, DREADD-mediated strategy can be expanded by coupling "classical" muscarinic receptor mutants with the recently developed inhibitory kappa-opioid DREADD that is activated by salvinorin B instead of CNO (Vardy et al. 2015). With this strategy two different neuronal populations can be inhibited or activated and inhibited at the same time or independently (Rapanelli et al. 2017). A similar approach could rely on the use of "PSAM-PSEM" actuators (see for review Sternson and Roth 2014). Moreover a similar experimental design can be drawn with optogenetics using the combination of both inhibitory and excitatory channels (Kleinlogel et al. 2011; Carter et al. 2012) and the use of red-shifted mutants of channelrhodopsin or halorhodopsin (Chow et al. 2010; Chuong et al. 2014; Klapoetke et al. 2014). On top of this profusion of various actuators, elegant genetic tagging of multiple neuronal subpopulations can be achieved through the use of intersectional strategies in transgenic animals expressing multiple recombination enzymes such as cre and flippase, under the control of different promoters (Fenno et al. 2014; He et al. 2016). Although challenging, this approach could be even expanded by adding an extra layer of control with the use of tetracycline-controlled transcriptional activation systems, which can be reversibly turned on and off in the presence of doxycycline (Zhang et al. 2015). And obviously, genetically encoded actuators can be

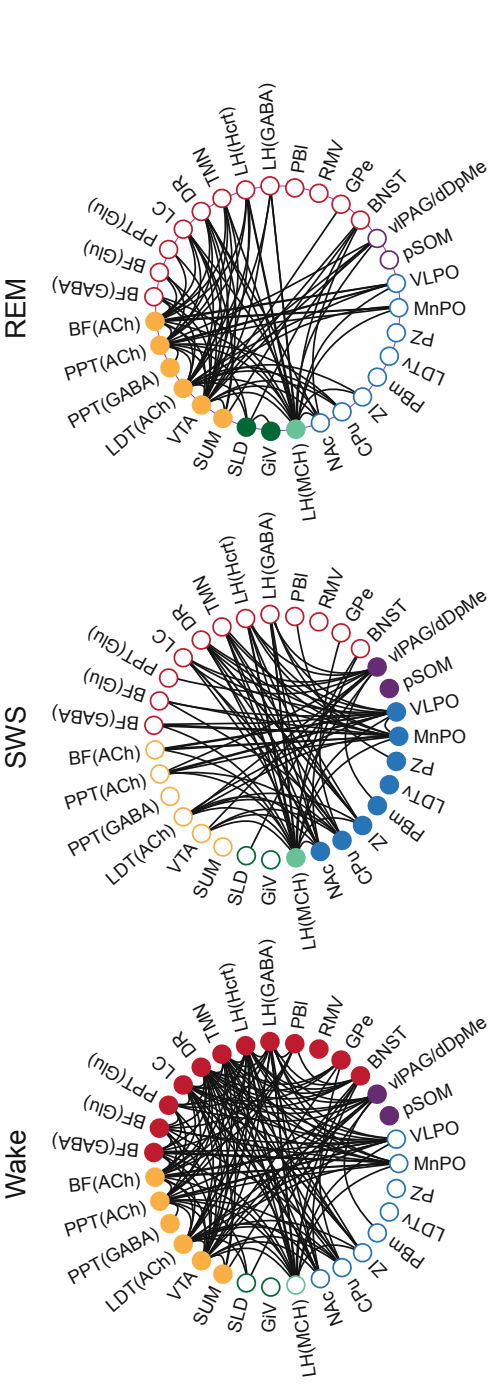

Fig. 2 Circular node diagrams of sleep/wake network connectivity including chemogenetic findings. Note that wake-related nuclei show high level of connectivity with sleeping state-related structures and a strong interconnectivity, which highlights their interdependency as well as their conjoint and reinforcing arousing actions. In contrast, formerly identified sleep-promoting nuclei and newly chemogenetically identified structures associated with either SWS or REM sleep both display important connections with the wake-promoting systems but barely share connections among them. At our current state of knowledge, the latter characteristics would imply multiple and independent nodes promoting and regulating sleep states. Alternatively, future investigations should further focus on identifying direct links between sleep-regulating structures

multiplexed with genetically encoded sensors such as calcium indicators or voltage-sensitive fluorescent proteins (Kim et al. 2016).

Finally, the complexity of sleep physiology and the complexity of neuronal networks on which it depends should inspire the use of more and more sophisticated metrics to acutely and precisely dissect sleep-wake architecture and dynamics, together with subtle regulatory mechanisms. Among these metrics can be mentioned Markov's chain analysis (Kim et al. 2009; Bianchi et al. 2012; Stephenson et al. 2013), survival analysis of sleep and wake episode duration (Lo et al. 2002, 2004; Blumberg et al. 2005; Diniz Behn et al. 2008; Varin et al. 2016), or EEG or LFP analysis using two dimensional map of behavioral state (Gervasoni et al. 2004; Dzirasa et al. 2006; Diniz Behn et al. 2010). All together, the above methods should provide new ways to analyze and decipher the precise temporal, hierarchical, and dynamical organization of vigilance states.

References

Adamantidis A, Lüthi A (2018) Optogenetic dissection of sleep-wake states in vitro and in vivo. In: Handbook of experimental pharmacology. Springer, Berlin/Heidelberg. https://doi.org/10.1007/164_2018_94

Adamantidis AR, Zhang F, Aravanis AM, Deisseroth K, de Lecea L (2007) Neural substrates of awakening probed with optogenetic control of hypocretin neurons. Nature 450:420–424

Alcacer C, Andreoli L, Sebastianutto I, Jakobsson J, Fieblinger T, Cenci MA (2017) Chemogenetic stimulation of striatal projection neurons modulates responses to Parkinson's disease therapy. J Clin Invest 127:720–734

Alexander GM, Rogan SC, Abbas AI, Armbruster BN, Pei Y, Allen JA, Nonneman RJ, Hartmann J, Moy SS, Nicolelis MA, McNamara JO, Roth BL (2009) Remote control of neuronal activity in transgenic mice expressing evolved G protein-coupled receptors. Neuron 63:27–39

Ambrosini MV, Giuditta A (2001) Learning and sleep: the sequential hypothesis. Sleep Med Rev 5:477–490

Anaclet C, Fuller PM (2017) Brainstem regulation of slow-wave-sleep. Curr Opin Neurobiol 44:139–143

Anaclet C, Parmentier R, Ouk K, Guidon G, Buda C, Sastre J-P, Akaoka H, Sergeeva OA, Yanagisawa M, Ohtsu H, Franco P, Haas HL, Lin JS (2009) Orexin/hypocretin and histamine: distinct roles in the control. J Neurosci 29:14423–14438

Anaclet C, Lin J-S, Vetrivelan R, Krenzer M, Vong L, Fuller PM, Lu J (2012) Identification and characterization of a sleep-active cell group in the rostral medullary brainstem. J Neurosci 32:17970–17976

Anaclet C, Ferrari L, Arrigoni E, Bass CE, Saper CB, Lu J, Fuller PM (2014) The GABAergic parafacial zone is a medullary slow wave sleep-promoting center. Nat Neurosci 17:1217–1224

Anaclet C, Pedersen NP, Ferrari LL, Venner A, Bass CE, Arrigoni E, Fuller PM (2015) Basal forebrain control of wakefulness and cortical rhythms. Nat Commun 6:1–14

Armbruster BN, Li X, Pausch MH, Herlitze S, Roth BL (2007) Evolving the lock to fit the key to create a family of G protein-coupled receptors potently activated by an inert ligand. Proc Natl Acad Sci U S A 104:5163–5168

Aston-Jones G, Bloom FE (1981) Activity of norepinephrine-containing locus coeruleus neurons in behaving rats anticipates fluctuations in the sleep-waking cycle. J Neurosci 1:876–886

Aston-Jones G, Rajkowski J, Cohen J (1999) Role of locus coeruleus in attention and behavioral flexibility. Biol Psychiatry 46:1309–1320

Balleine BW, O'Doherty JP (2010) Human and rodent homologies in action control: corticostriatal determinants of goal-directed and habitual action. Neuropsychopharmacology 35:48–69
Bauer EP (2015) Serotonin in fear conditioning processes. Behav Brain Res 277:68–77
Bender D, Holschbach M, Stöcklin G (1994) Synthesis of n.c.a. carbon-11 labelled clozapine and its major metabolite clozapine-N-oxide and comparison of their biodistribution in mice. Nucl Med Biol 21:921–925
Berridge CW (2008) Noradrenergic modulation of arousal. Brain Res Rev 58:1–17
Bester H, Besson JM, Bernard JF (1997) Organization of efferent projections from the parabrachial area to the hypothalamus: a Phaseolus vulgaris-leucoagglutinin study in the rat. J Comp Neurol 383:245–281
Bianchi MT, Eiseman NA, Cash SS, Mietus J, Peng CK, Thomas RJ (2012) Probabilistic sleep architecture models in patients with and without sleep apnea. J Sleep Res 21:330–341
Billwiller F, Renouard L, Clement O, Fort P, Luppi PH (2017) Differential origin of the activation of dorsal and ventral dentate gyrus granule cells during paradoxical (REM) sleep in the rat. Brain Struct Funct 222:1495–1507
Bjorness TE, Greene RW (2009) Adenosine and sleep. Curr Neuropharmacol 7:238–245
Blandina P, Munari L, Provensi G, Passani MB (2012) Histamine neurons in the tuberomamillary nucleus: a whole center or distinct subpopulations? Front Syst Neurosci 6:33
Blumberg MS, Seelke AMH, Lowen SB, Karlsson KAE (2005) Dynamics of sleep-wake cyclicity in developing rats. Proc Natl Acad Sci U S A 102:14860–14864
Bolam JP, Ellender TJ (2016) Histamine and the striatum. Neuropharmacology 106:74–84
Bonnavion P, De Lecea L (2010) Hypocretins in the control of sleep and wakefulness. Curr Neurol Neurosci Rep 10:174–179
Bonnavion P, Jackson AC, Carter ME, de Lecea L (2015) Antagonistic interplay between hypocretin and leptin in the lateral hypothalamus regulates stress responses. Nat Commun 6:6266
Bonnavion P, Mickelsen LE, Fujita A, de Lecea L, Jackson AC (2016) Hubs and spokes of the lateral hypothalamus: cell types, circuits and behaviour. J Physiol 594:6443–6462
Boucetta S, Cisse Y, Mainville L, Morales M, Jones BE (2014) Discharge profiles across the sleep-waking cycle of identified cholinergic, GABAergic, and glutamatergic neurons in the pontomesencephalic tegmentum of the rat. J Neurosci 34:4708–4727
Boyce R, Glasgow SD, Williams S, Adamantidis A (2016) Sleep research: causal evidence for the role of REM sleep theta rhythm in contextual memory consolidation. Science 352:812–816
Brancaccio M, Maywood ES, Chesham JE, Loudon ASI, Hastings MH (2013) A Gq-Ca2+ axis controls circuit-level encoding of circadian time in the suprachiasmatic nucleus. Neuron 78:714–728
Brown RE, McKenna JT (2015) Turning a negative into a positive: ascending GABAergic control of cortical activation and arousal. Front Neurol 6:135
Brown RE, Basheer R, McKenna JT, Strecker RE, McCarley RW (2012) Control of sleep and wakefulness. Physiol Rev 92:1087–1187
Buzsáki G, Schomburg EW (2015) What does gamma coherence tell us about inter-regional neural communication? Nat Neurosci 18:484–489
Carter ME, Yizhar O, Chikahisa S, Nguyen H, Adamantidis A, Nishino S, Deisseroth K, de Lecea L (2010) Tuning arousal with optogenetic modulation of locus coeruleus neurons. Nat Neurosci 13:1526–1533
Carter ME, Brill J, Bonnavion P, Huguenard JR, Huerta R, de Lecea L (2012) Mechanism for hypocretin-mediated sleep-to-wake transitions. Proc Natl Acad Sci U S A 109:E2635–E2644
Carvalho Poyraz F, Holzner E, Bailey MR, Meszaros J, Kenney L, Kheirbek MA, Balsam PD, Kellendonk C (2016) Decreasing striatopallidal pathway function enhances motivation by energizing the initiation of goal-directed action. J Neurosci 36:5988–6001
Chang WH, Lin SK, Lane HY, Wei FC, Hu WH, Lam YWF, Jann MW (1998) Reversible metabolism of clozapine and clozapine N-oxide in schizophrenic patients. Prog Neuropsychopharmacol Biol Psychiatry 22:723–739

Chen X, Choo H, Huang XP, Yang X, Stone O, Roth BL, Jin J (2015) The first structure-activity relationship studies for designer receptors exclusively activated by designer drugs. ACS Chem Nerosci 6:476–484

Chen L, Yin D, Wang TX, Guo W, Dong H, Xu Q, Luo YJ, Cherasse Y, Lazarus M, Qiu ZL, Lu J, Qu WM, Huang ZL (2016) Basal forebrain cholinergic neurons primarily contribute to inhibition of electroencephalogram delta activity, rather than inducing behavioral wakefulness in mice. Neuropsychopharmacology 41:2133–2146

Chen MC, Vetrivelan R, Guo CN, Chang C, Fuller PM, Lu J (2017) Ventral medullary control of rapid eye movement sleep and atonia. Exp Neurol 290:53–62

Chow BY, Han X, Dobry AS, Qian X, Chuong AS, Li M, Henninger MA, Belfort GM, Lin Y, Monahan PE, Boyden ES (2010) High-performance genetically targetable optical neural silencing by light-driven proton pumps. Nature 463:98–102

Chung S, Weber F, Zhong P, Tan CL, Nguyen TN, Beier KT, Hörmann N, Chang WC, Zhang Z, Do JP, Yao S, Krashes MJ, Tasic B, Cetin A, Zeng H, Knight ZA, Luo L, Dan Y (2017) Identification of preoptic sleep neurons using retrograde labelling and gene profiling. Nature 545:477–481

Chuong AS, Miri ML, Busskamp V, Matthews GA, Acker LC, Sorensen AT, Young A, Klapoetke NC, Henninger MA, Kodandaramaiah SB, Ogawa M, Ramanial SB, Bandler RC, Allen BD, Forest CR, Chow BY, Han X, Lin Y, Tye KM, Roska B, Cardin JA, Boyden ES (2014) Noninvasive optical inhibition with a red-shifted microbial rhodopsin. Nat Neurosci 17:1123–1129

Conklin BR, Hsiao EC, Claeysen S, Dumuis A, Srinivasan S, Forsayeth JR, Guettier JM, Chang WC, Pei Y, McCarthy KD, Nissenson RA, Wess J, Bockaert J, Roth BL (2008) Engineering GPCR signaling pathways with RASSLs. Nat Methods 5:673–678

Dahan L, Astier B, Vautrelle N, Urbain N, Kocsis B, Chouvet G (2007) Prominent burst firing of dopaminergic neurons in the ventral tegmental area during paradoxical sleep. Neuropsychopharmacology 32:1232–1241

Datta S, Mavanji V, Ulloor J, Patterson EH (2004) Activation of phasic pontine-wave generator prevents rapid eye movement sleep deprivation-induced learning impairment in the rat: a mechanism for sleep-dependent plasticity. J Neurosci 24:1416–1427

Dauvilliers Y, Billiard M, Montplaisir J (2003) Clinical aspects and pathophysiology of narcolepsy. Clin Neurophysiol 114:2000–2017

De Lecea L (2012) Hypocretins and the neurobiology of sleep-wake mechanisms. Prog Brain Res 198:15–24

Diniz Behn CG, Kopell N, Brown EN, Mochizuki T, Scammell TE (2008) Delayed orexin signaling consolidates wakefulness and sleep: physiology and modeling. J Neurophysiol 99:3090–3103

Diniz Behn CG, Klerman EB, Mochizuki T, Lin SC, Scammell TE (2010) Abnormal sleep/wake dynamics in orexin knockout mice. Sleep 33:297–306

Dong S, Rogan SC, Roth BL (2010) Directed molecular evolution of DREADDs: a generic approach to creating next-generation RASSLs. Nat Protoc 5:561–573

Drews J (2000) Drug discovery: a historical perspective. Science 287:1960–1964

Durieux PF, Schiffmann SN, de Kerchove d'Exaerde A (2011) Targeting neuronal populations of the striatum. Front Neuroanat 5:40

Dzirasa K, Ribeiro S, Costa R, Santos LM, Lin S-C, Grosmark A, Sotnikova TD, Gainetdinov RR, Caron MG, Nicolelis MAL (2006) Dopaminergic control of sleep-wake states. J Neurosci 26:10577–10589

Eban-Rothschild A, Rothschild G, Giardino WJ, Jones JR, de Lecea L (2016) VTA dopaminergic neurons regulate ethologically relevant sleep-wake behaviors. Nat Neurosci 19(10):1356–1366

Economo C (1930) Sleep as a problem of localization. J Nerv Ment Dis 71:249–259

Eldridge MAG, Lerchner W, Saunders RC, Kaneko H, Krausz KW, Gonzalez FJ, Ji B, Higuchi M, Minamimoto T, Richmond BJ (2015) Chemogenetic disconnection of monkey orbitofrontal and rhinal cortex reversibly disrupts reward value. Nat Neurosci 19:37–39

Farrell MS, Roth BL (2013) Pharmacosynthetics: reimagining the pharmacogenetic approach. Brain Res 1511:6–20

Farrell MS, Pei Y, Wan Y, Yadav PN, Daigle TL, Urban DJ, Lee H-M, Sciaky N, Simmons A, Nonneman RJ, Huang X-P, Hufeisen SJ, Guettier J-M, Moy SS, Wess J, Caron MG, Calakos N, Roth BL (2013) A Gαs DREADD mouse for selective modulation of cAMP production in striatopallidal neurons. Neuropsychopharmacology 38:854–862

Fenno LE, Mattis J, Ramakrishnan C, Hyun M, Lee SY, He M, Tucciarone J, Selimbeyoglu A, Berndt A, Grosenick L, Zalocusky KA, Bernstein H, Swanson H, Perry C, Diester I, Boyce FM, Bass CE, Neve R, Huang ZJ, Deisseroth K (2014) Targeting cells with single vectors using multiple-feature Boolean logic. Nat Methods 11:763–772

Fort P, Bassetti CL, Luppi PH (2009) Alternating vigilance states: new insights regarding neuronal networks and mechanisms. Eur J Neurosci 29:1741–1753

Fortress AM, Hamlett ED, Vazey EM, Aston-Jones G, Cass WA, Boger HA, Granholm A-CE (2015) Designer receptors enhance memory in a mouse model of down syndrome. J Neurosci 35:1343–1353

Fraigne JJ, Torontali ZA, Snow MB, Peever JH (2015) REM sleep at its core-circuits, neurotransmitters, and pathophysiology. Front Neurol 6:123

Fujita A, Bonnavion P, Wilson MH, Mickelsen LE, Bloit J, de Lecea L, Jackson AC (2017) Hypothalamic tuberomammillary nucleus neurons: electrophysiological diversity and essential role in arousal stability. J Neurosci 37:9574–9592

Fuller P, Sherman D, Pedersen NP, Saper CB, Lu J (2011) Reassessment of the structural basis of the ascending arousal system. J Comp Neurol 519:933–956

Gervasoni D, Lin SC, Ribeiro S, Soares ES, Pantoja J, Nicolelis MA (2004) Global forebrain dynamics predict rat behavioral states and their transitions. J Neurosci 24:11137–11147

Giuditta A (2014) Sleep memory processing: the sequential hypothesis. Front Syst Neurosci 8:219

Gomez JL, Bonaventura J, Lesniak W, Mathews WB, Sysa-Shah P, Rodriguez LA, Ellis RJ, Richie CT, Harvey BK, Dannals RF, Pomper MG, Bonci A, Michaelides M (2017) Chemogenetics revealed: DREADD occupancy and activation via converted clozapine. Science 357:503–507

González JA, Iordanidou P, Strom M, Adamantidis A, Burdakov D (2016) Awake dynamics and brain-wide direct inputs of hypothalamic MCH and orexin networks. Nat Commun 7:11395

Graybiel AM (2008) Habits, rituals, and the evaluative brain. Annu Rev Neurosci 31:359–387

Grivel J, Cvetkovic V, Bayer L, Machard D, Tobler I, Muhlethaler M, Serafin M (2005) The wake-promoting hypocretin/orexin neurons change their response to noradrenaline after sleep deprivation. J Neurosci 25:4127–4130

Guettier J-M, Gautam D, Scarselli M, Ruiz de Azua I, Li JH, Rosemond E, Ma X, Gonzalez FJ, Armbruster BN, Lu H, Roth BL, Wess J (2009) A chemical-genetic approach to study G protein regulation of beta cell function in vivo. Proc Natl Acad Sci U S A 106:19197–19202

Haas HL, Sergeeva OA, Selbach O (2008) Histamine in the nervous system. Physiol Rev 88:1183–1241

Han Y, Shi YF, Xi W, Zhou R, Tan ZB, Wang H, Li XM, Chen Z, Feng G, Luo M, Huang ZL, Duan S, Yu YQ (2014) Selective activation of cholinergic basal forebrain neurons induces immediate sleep-wake transitions. Curr Biol 24:693–698

Hara J, Beuckmann CT, Nambu T, Willie JT, Chemelli RM, Sinton CM, Sugiyama F, Yagami KI, Goto K, Yanagisawa M, Sakurai T (2001) Genetic ablation of orexin neurons in mice results in narcolepsy, hypophagia, and obesity. Neuron 30:345–354

Hasegawa E, Yanagisawa M, Sakurai T, Mieda M (2014) Orexin neurons suppress narcolepsy via 2 distinct efferent pathways. J Clin Invest 124:604–616

Hasegawa E, Maejima T, Yoshida T, Masseck OA, Herlitze S, Yoshioka M, Sakurai T, Mieda M (2017) Serotonin neurons in the dorsal raphe mediate the anticataplectic action of orexin neurons by reducing amygdala activity. Proc Natl Acad Sci U S A 114:E3526–E3535

Hassani OK, Henny P, Lee MG, Jones BE (2010) GABAergic neurons intermingled with orexin and MCH neurons in the lateral hypothalamus discharge maximally during sleep. Eur J Neurosci 32:448–457

Hayashi Y, Kashiwagi M, Yasuda K, Ando R, Kanuka M, Sakai K, Itohara S (2015) Cells of a common developmental origin regulate REM/non-REM sleep and wakefulness in mice. Science 350:957–962

He M, Tucciarone J, Lee S, Nigro MJ, Kim Y, Levine JM, Kelly SM, Krugikov I, Wu P, Chen Y, Gong L, Hou Y, Osten P, Rudy B, Huang ZJ (2016) Strategies and tools for combinatorial targeting of GABAergic neurons in mouse cerebral cortex. Neuron 91:1228–1243

Hellman K, Aadal Nielsen P, Ek F, Olsson R (2016) An ex vivo model for evaluating blood-brain barrier permeability, efflux, and drug metabolism. ACS Chem Nerosci 7:668–680

Herrera CG, Cadavieco MC, Jego S, Ponomarenko A, Korotkova T, Adamantidis A (2015) Hypothalamic feedforward inhibition of thalamocortical network controls arousal and consciousness. Nat Neurosci 19:1–12

Herrera CG, Ponomarenko A, Korotkova T, Burdakov D, Adamantidis A (2017) Sleep and metabolism: the multitasking ability of lateral hypothalamic inhibitory circuitries. Front Neuroendocrinol 44:27–34

Hinze-Selch D, Mullington J, Orth A, Lauer CJ, Pollmächer T (1997) Effects of clozapine on sleep: a longitudinal study. Biol Psychiatry 42:260–266

Inostroza M, Binder S, Born J (2013) Sleep-dependency of episodic-like memory consolidation in rats. Behav Brain Res 237:15–22

Janak PH, Tye KM (2015) From circuits to behaviour in the amygdala. Nature 517:284–292

Jann MW, Lam YW, Chang WH (1994) Rapid formation of clozapine in guinea-pigs and man following clozapine-N-oxide administration. Arch Int Pharmacodyn Ther 328:243–250

Jego S, Glasgow SD, Herrera CG, Ekstrand M, Reed SJ, Boyce R, Friedman J, Burdakov D, Adamantidis AR (2013) Optogenetic identification of a rapid eye movement sleep modulatory circuit in the hypothalamus. Nat Neurosci 16:1637–1643

Jennings JH, Rizzi G, Stamatakis AM, Ung RL, Stuber GD (2013) The inhibitory circuit architecture of the lateral hypothalamus orchestrates feeding. Science 341:1517–1521

Ji B, Kaneko H, Minamimoto T, Inoue H, Takeuchi H, Kumata K, Zhang M-R, Aoki I, Seki C, Ono M, Tokunaga M, Tsukamoto S, Tanabe K, Shin R-M, Minamihisamatsu T, Kito S, Richmond BJ, Suhara T, Higuchi M (2016) Multimodal imaging for DREADD-expressing neurons in living brain and their application to implantation of iPSC-derived neural progenitors. J Neurosci 36:11544–11558

Jin X, Costa RM (2010) Start/stop signals emerge in nigrostriatal circuits during sequence learning. Nature 466:457–462

Jouvet M (1999) Sleep and serotonin: an unfinished story. Neuropsychopharmacology 21:24S–27S

Kantor S, Mochizuki T, Janisiewicz AM, Clark E, Nishino S, Scammell TE (2009) Orexin neurons are necessary for the circadian control of REM sleep. Sleep 32:1127–1134

Kaur S, Wang JL, Ferrari L, Thankachan S, Kroeger D, Venner A, Lazarus M, Wellman A, Arrigoni E, Fuller PM, Saper CB (2017) A genetically defined circuit for arousal from sleep during hypercapnia. Neuron 96:1153–1167.e5

Kim JW, Lee JS, Robinson PA, Jeong DU (2009) Markov analysis of sleep dynamics. Phys Rev Lett 102:178104

Kim T, Thankachan S, McKenna JT, McNally JM, Yang C, Choi JH, Chen L, Kocsis B, Deisseroth K, Strecker RE, Basheer R, Brown RE, McCarley RW (2015) Cortically projecting basal forebrain parvalbumin neurons regulate cortical gamma band oscillations. Proc Natl Acad Sci U S A 112:3535–3540

Kim CK, Yang SJ, Pichamoorthy N, Young NP, Kauvar I, Jennings JH, Lerner TN, Berndt A, Lee SY, Ramakrishnan C, Davidson TJ, Inoue M, Bito H, Deisseroth K (2016) Simultaneous fast measurement of circuit dynamics at multiple sites across the mammalian brain. Nat Methods 13:325–328

Klapoetke NC et al (2014) Independent optical excitation of distinct neural populations. Nat Methods 11:338–346

Kleinlogel S, Terpitz U, Legrum B, Gökbuget D, Boyden ES, Bamann C, Wood PG, Bamberg E (2011) A gene-fusion strategy for stoichiometric and co-localized expression of light-gated membrane proteins. Nat Methods 8:1083–1091

Kobilka BK, Deupi X (2007) Conformational complexity of G-protein-coupled receptors. Trends Pharmacol Sci 28:397–406

Kodani S, Soya S, Sakurai T (2017) Excitation of GABAergic neurons in the bed nucleus of the stria terminalis triggers immediate transition from non-rapid eye movement sleep to wakefulness in mice. J Neurosci 37:7164–7176

Konadhode RR, Pelluru D, Blanco-Centurion C, Zayachkivsky A, Liu M, Uhde T, Glen WB, van den Pol AN, Mulholland PJ, Shiromani PJ (2013) Optogenetic stimulation of MCH neurons increases sleep. J Neurosci 33:10257–10263

Kosse C, Burdakov D (2014) A unifying computational framework for stability and flexibility of arousal. Front Syst Neurosci 8:192

Kosse C, Schöne C, Bracey E, Burdakov D (2017) Orexin-driven GAD65 network of the lateral hypothalamus sets physical activity in mice. Proc Natl Acad Sci U S A 114:4525–4530

Krashes MJ, Koda S, Ye CP, Rogan SC, Adams AC, Cusher DS, Maratos-Flier E, Roth BL, Lowell BB (2011) Rapid, reversible activation of AgRP neurons drives feeding behavior in mice. J Clin Invest 121:1424–1428

Kroeger D, Ferrari LL, Petit G, Mahoney CE, Fuller PM, Arrigoni E, Scammell TE (2017) Cholinergic, glutamatergic, and GABAergic neurons of the pedunculopontine tegmental nucleus have distinct effects on sleep/wake behavior in mice. J Neurosci 37:1352–1366

Lazarus M, Huang ZL, Lu J, Urade Y, Chen JF (2012) How do the basal ganglia regulate sleep-wake behavior? Trends Neurosci 35:723–732

Lebow MA, Chen A (2016) Overshadowed by the amygdala: the bed nucleus of the stria terminalis emerges as key to psychiatric disorders. Mol Psychiatry 21:450–463

Léna I, Parrot S, Deschaux O, Muffat-Joly S, Sauvinet V, Renaud B, Suaud-Chagny MF, Gottesmann C (2005) Variations in extracellular levels of dopamine, noradrenaline, glutamate, and aspartate across the sleep-wake cycle in the medial prefrontal cortex and nucleus accumbens of freely moving rats. J Neurosci Res 81:891–899

Lin JS (2000) Brain structures and mechanisms involved in the control of cortical activation and wakefulness, with emphasis on the posterior hypothalamus and histaminergic neurons. Sleep Med Rev 4:471–503

Lin JS, Sergeeva OA, Haas HL (2011a) Histamine H3 receptors and sleep-wake regulation. J Pharmacol Exp Ther 336:17–23

Lin JS, Anaclet C, Sergeeva OA, Haas HL (2011b) The waking brain: an update. Cell Mol Life Sci 68:2499–2512

Liu K, Kim J, Kim DW, Zhang YS, Bao H, Denaxa M, Lim SA, Kim E, Liu C, Wickersham IR, Pachinis V, Hattar S, Song J, Brown SP, Blackshaw S (2017) Lhx6-positive GABA-releasing neurons of the zona incerta promote sleep. Nature 548:582–587

Llinas RR, Steriade M (2006) Bursting of thalamic neurons and states of vigilance. J Neurophysiol 95:3297–3308

Lo CC, Amaral LAN, Havlin S, Ivanov PC, Penzel T, Peter JH, Stanley HE (2002) Dynamics of sleep-wake transitions during sleep. Europhys Lett 57:625–631

Lo C-C, Chou T, Penzel T, Scammell TE, Strecker RE, Stanley HE, Ivanov PC (2004) Common scale-invariant patterns of sleep-wake transitions across mammalian species. Proc Natl Acad Sci U S A 101:17545–17548

Loffler S, Korber J, Nubbemeyer U, Fehsel K (2012) Comment on "Impaired respiratory and body temperature control upon acute serotonergic neuron inhibition". Science 337:646–646

Lőrincz ML, Adamantidis AR (2017) Monoaminergic control of brain states and sensory processing: existing knowledge and recent insights obtained with optogenetics. Prog Neurobiol 151:237–253

Luppi PH, Peyron C, Fort P (2017) Not a single but multiple populations of GABAergic neurons control sleep. Sleep Med Rev 32:85–94

MacLaren DA, Browne RW, Shaw JK, Krishnan Radhakrishnan S, Khare P, Espana RA, Clark SD (2016) Clozapine N-oxide administration produces behavioral effects in long-evans rats: implications for designing DREADD experiments. eNeuro 3

Magill PJ, Bolam JP, Bevan MD (2000) Relationship of activity in the subthalamic nucleus-globus pallidus network to cortical electroencephalogram. J Neurosci 20:820–833

Mahler SV, Aston-Jones G (2018) CNO Evil? considerations for the use of DREADDs in behavioral neuroscience. Neuropsychopharmacology 43:934–936

Mahon S, Vautrelle N, Pezard L, Slaght SJ, Deniau JM, Chouvet G, Charpier S (2006) Distinct patterns of striatal medium spiny neuron activity during the natural sleep-wake cycle. J Neurosci 26:12587–12595

Mandile P, Vescia S, Montagnese P, Romano F, Giuditta A (1996) Characterization of transition sleep episodes in baseline EEG recordings of adult rats. Physiol Behav 60:1435–1439

Maudhuit C, Jolas T, Lainey E, Hamon M, Adrien J (1994) Effects of acute and chronic treatment with amoxapine and cericlamine on the sleep-wakefulness cycle in the rat. Neuropharmacology 33:1017–1025

McCall JG, Al-Hasani R, Siuda ER, Hong DY, Norris AJ, Ford CP, Bruchas MR (2015) CRH engagement of the locus coeruleus noradrenergic system mediates stress-induced anxiety. Neuron 87:606–621

McGinty DJ, Harper RM (1976) Dorsal raphe neurons: depression of firing during sleep in cats. Brain Res 101:569–575

Mileykovskiy BY, Kiyashchenko LI, Siegel JM (2005) Behavioral correlates of activity in identified hypocretin/orexin neurons. Neuron 46:787–798

Miller JD, Farber J, Gatz P, Roffwarg H, German DC (1983) Activity of mesencephalic dopamine and non-dopamine neurons across stages of sleep and waking in the rat. Brain Res 273:133–141

Monaca C, Boutrel B, Hen R, Hamon M, Adrien J (2003) 5-HT1A/1B receptor-mediated effects of the selective serotonin reuptake inhibitor, citalopram, on sleep: studies in 5-HT1A and 5-HT1B knockout mice. Neuropsychopharmacol 28:850–856

Nagai Y, Kikuchi E, Lerchner W, Inoue KI, Ji B, Eldridge MA, Kaneko H, Kimura Y, Oh-Nishi A, Hori Y, Kato Y, Hirabayashi T, Fujimoto A, Kumata K, Zhang MR, Aoki I, Suhara T, Higuchi M, Takada M, Richmond BJ, Minamimoto T (2016) PET imaging-guided chemogenetic silencing reveals a critical role of primate rostromedial caudate in reward evaluation. Nat Commun 7:13605

Nakajima K, Wess J (2012) Design and functional characterization of a novel, arrestin-biased designer G protein-coupled receptor. Mol Pharmacol 82:575–582

Nishida M, Pearsall J, Buckner RL, Walker MP (2009) REM sleep, prefrontal theta, and the consolidation of human emotional memory. Cereb Cortex 19:1158–1166

Oishi Y, Xu Q, Wang L, Zhang BJ, Takahashi K, Takata Y, Luo YJ, Cherasse Y, Schiffmann SN, De Kerchove D'Exaerde A, Urade Y, Qu WM, Huang ZL, Lazarus M (2017) Slow-wave sleep is controlled by a subset of nucleus accumbens core neurons in mice. Nat Commun 8:734

Orzeł-Gryglewska J, Matulewicz P, Jurkowlaniec E (2015) Brainstem system of hippocampal theta induction: the role of the ventral tegmental area. Synapse 69:553–575

Pan WX, McNaughton N (2004) The supramammillary area: its organization, functions and relationship to the hippocampus. Prog Neurobiol 74:127–166

Parmentier R, Ohtsu H, Djebbara-Hannas Z, Valatx J-L, Watanabe T, Lin J-S (2002) Anatomical, physiological, and pharmacological characteristics of histidine decarboxylase knock-out mice: evidence for the role of brain histamine in behavioral and sleep-wake control. J Neurosci 22:7695–7711

Parnaudeau S, O'Neill PK, Bolkan SS, Ward RD, Abbas AI, Roth BL, Balsam PD, Gordon JA, Kellendonk C (2013) Inhibition of mediodorsal thalamus disrupts thalamofrontal connectivity and cognition. Neuron 77:1151–1162

Pedersen NP, Ferrari L, Venner A, Wang JL, Abbott SBG, Vujovic N, Arrigoni E, Saper CB, Fuller PM (2017) Supramammillary glutamate neurons are a key node of the arousal system. Nat Commun 8:1405

Popa D, Duvarci S, Popescu AT, Lena C, Pare D (2010) Coherent amygdalocortical theta promotes fear memory consolidation during paradoxical sleep. Proc Natl Acad Sci U S A 107:6516–6519

Portas CM, Bjorvatn B, Ursin R (2000) Serotonin and the sleep/wake cycle: special emphasis on microdialysis studies. Prog Neurobiol 60:12–35

Qiu MH, Chen MC, Fuller PM, Lu J (2016a) Stimulation of the pontine parabrachial nucleus promotes wakefulness via extra-thalamic forebrain circuit nodes. Curr Biol 26:2301–2312

Qiu MH, Yao QL, Vetrivelan R, Chen MC, Lu J (2016b) Nigrostriatal dopamine acting on globus pallidus regulates sleep. Cereb Cortex 26:1430–1439

Rapanelli M, Frick L, Bito H, Pittenger C (2017) Histamine modulation of the basal ganglia circuitry in the development of pathological grooming. Proc Natl Acad Sci U S A 114:6599–6604

Raper J, Morrison RD, Daniels JS, Howell L, Bachevalier J, Wichmann T, Galvan A (2017) Metabolism and distribution of clozapine-N-oxide: implications for nonhuman primate chemogenetics. ACS Chem Nerosci 8:1570–1576

Rasmussen K, Heym J, Jacobs BL (1984) Activity of serotonin-containing neurons in nucleus centralis superior of freely moving cats. Exp Neurol 83:302–317

Ravassard P, Hamieh AM, Joseph MA, Fraize N, Libourel PA, Lebarillier L, Arthaud S, Meissirel C, Touret M, Malleret G, Salin PA (2016) REM sleep-dependent bidirectional regulation of hippocampal-based emotional memory and LTP. Cereb Cortex 26:1488–1500

Ray RS, Corcoran AE, Brust RD, Kim JC, Richerson GB, Nattie E, Dymecki SM (2011) Impaired respiratory and body temperature control upon acute serotonergic neuron inhibition. Science 333:637–642

Reijmers LG, Perkins BL, Matsuo N, Mayford M (2007) Localization of a stable neural correlate of associative memory. Science 317:1230–1233

Reiner PB, McGeer EG (1987) Electrophysiological properties of cortically projecting histamine neurons of the rat hypothalamus. Neurosci Lett 73:43–47

Renouard L, Billwiller F, Ogawa K, Clément O, Camargo N, Abdelkarim M, Gay N, Scoté-Blachon C, Touré R, Libourel PA, Ravassard P, Salvert D, Peyron C, Claustrat B, Léger L, Salin P, Malleret G, Fort P, Luppi PH (2015) The supramammillary nucleus and the claustrum activate the cortex during REM sleep. Sci Adv 1:e1400177

Rioult-Pedotti MS, Pekanovic A, Atiemo CO, Marshall J, Luft AR (2015) Dopamine promotes motor cortex plasticity and motor skill learning via PLC activation. PLoS One 10:e0124986

Rolls A, Colas D, Adamantidis A, Carter M, Lanre-Amos T, Heller HC, de Lecea L (2011) Optogenetic disruption of sleep continuity impairs memory consolidation. Proc Natl Acad Sci U S A 108:13305–13310

Root DH, Melendez RI, Zaborszky L, Napier TC (2015) The ventral pallidum: subregion-specific functional anatomy and roles in motivated behaviors. Prog Neurobiol 130:29–70

Rorabaugh JM, Chalermpalanupap T, Botz-Zapp CA, Fu VM, Lembeck NA, Cohen RM, Weinshenker D (2017) Chemogenetic locus coeruleus activation restores reversal learning in a rat model of Alzheimer's disease. Brain 140:3023–3038

Roth BL (2016) DREADDs for neuroscientists. Neuron 89:683–694

Saito YC, Tsujino N, Hasegawa E, Akashi K, Abe M, Mieda M, Sakimura K, Sakurai T (2013) GABAergic neurons in the preoptic area send direct inhibitory projections to orexin neurons. Front Neural Circuits 7:192

Sakai K (2011) Sleep-waking discharge profiles of dorsal raphe nucleus neurons in mice. Neuroscience 197:200–224

Sakai K, Takahashi K, Anaclet C, Lin J-S (2010) Sleep-waking discharge of ventral tuberomammillary neurons in wild-type and histidine decarboxylase knock-out mice. Front Behav Neurosci 4:53

Santos LM, Dzirasa K, Kubo R, Silva MTA, Ribeiro S, Sameshima K, Valle AC, Timo-Iaria C (2008) Baseline hippocampal theta oscillation speeds correlate with rate of operant task acquisition. Behav Brain Res 190:152–155

Sapin E, Lapray D, Bérod A, Goutagny R, Léger L, Ravassard P, Clément O, Hanriot L, Fort P, Luppi PH (2009) Localization of the brainstem GABAergic neurons controlling paradoxical (REM) sleep. PLoS One 4:e4272

Sara SJ (2009) The locus coeruleus and noradrenergic modulation of cognition. Nat Rev Neurosci 10:211–223

Sara SJ (2017) Sleep to remember dual perspectives. J Neurosci 37:457–463

Sasaki K, Suzuki M, Mieda M, Tsujino N, Roth B, Sakurai T (2011) Pharmacogenetic modulation of orexin neurons alters sleep/wakefulness states in mice. PLoS One 6:e20360

Satoh S, Matsumura H, Kanbayashi T, Yoshida Y, Urakami T, Nakajima T, Kimura N, Nishino S, Yoneda H (2006) Expression pattern of FOS in orexin neurons during sleep induced by an adenosine A2Areceptor agonist. Behav Brain Res 170:277–286

Schöne C, Burdakov D (2017) Orexin/hypocretin and organizing principles for a diversity of wake-promoting neurons in the brain. Curr Top Behav Neurosci 33:51–74

Schwartz JC (2011) The histamine H3 receptor: from discovery to clinical trials with pitolisant. Br J Pharmacol 163:713–721

Schwarz LA, Miyamichi K, Gao XJ, Beier KT, Weissbourd B, DeLoach KE, Ren J, Ibanes S, Malenka RC, Kremer EJ, Luo L (2015) Viral-genetic tracing of the input–output organization of a central noradrenaline circuit. Nature 524:88–92

Senba E, Daddona PE, Watanabe T, Wu JY, Nagy JI (1985) Coexistence of adenosine deaminase, histidine decarboxylase, and glutamate decarboxylase in hypothalamic neurons of the rat. J Neurosci 5:3393–3402

Slater IH, Jones GT, Moore RA (1978) Inhibition of REM sleep by fluoxetine, a specific inhibitor of serotonin uptake. Neuropharmacol 17:383–389

Sommerfelt L, Ursin R (1987) The effects of zimeldine and alaproclate combined with a small dose of 5-HTP on waking and sleep stages in cats. Behav Brain Res 24:1–10

Soussi R, Zhang N, Tahtakran S, Houser CR, Esclapez M (2010) Heterogeneity of the supramammillary-hippocampal pathways: evidence for a unique GABAergic neurotransmitter phenotype and regional differences. Eur J Neurosci 32:771–785

Stachniak TJ, Ghosh A, Sternson SM (2014) Chemogenetic synaptic silencing of neural circuits localizes a hypothalamus→midbrain pathway for feeding behavior. Neuron 82:797–808

Steinfels GF, Heym J, Strecker RE, Jacobs BL (1983) Behavioral correlates of dopaminergic unit activity in freely moving cats. Brain Res 258:217–228

Stephenson R, Famina S, Caron AM, Lim J (2013) Statistical properties of sleep-wake behavior in the rat and their relation to circadian and ultradian phases. Sleep 36:1377–1390

Steriade M (2004) Acetylcholine systems and rhythmic activities during the waking-sleep cycle. Prog Brain Res 145:179–196

Sternson SM, Roth BL (2014) Chemogenetic tools to interrogate brain functions. Annu Rev Neurosci 37:387–407

Sun HX, Wang DR, Ye CB, Hu ZZ, Wang CY, Huang ZL, Yang SR (2017) Activation of the ventral tegmental area increased wakefulness in mice. Sleep Biol Rhythms 15:107–115

Taheri S, Zeitzer JM, Mignot E (2002) The role of hypocretins (orexins) in sleep regulation and narcolepsy. Annu Rev Neurosci 25:283–313

Takahashi K, Lin J-S, Sakai K (2006) Neuronal activity of histaminergic tuberomammillary neurons during wake-sleep states in the mouse. J Neurosci 26:10292–10298

Takahashi K, Lin JS, Sakai K (2009) Characterization and mapping of sleep-waking specific neurons in the basal forebrain and preoptic hypothalamus in mice. Neuroscience 161:269–292

Takahashi K, Kayama Y, Lin JS, Sakai K (2010) Locus coeruleus neuronal activity during the sleep-waking cycle in mice. Neuroscience 169:1115–1126

Takeda N, Inagaki S, Shiosaka S, Taguchi Y, Oertel WH, Tohyama M, Watanabe T, Wada H (1984) Immunohistochemical evidence for the coexistence of histidine decarboxylase-like and glutamate decarboxylase-like immunoreactivities in nerve cells of the magnocellular nucleus of the posterior hypothalamus of rats. Proc Natl Acad Sci U S A 81:7647–7650

Thinschmidt JS, Kinney GG, Kocsis B (1995) The supramammillary nucleus: is it necessary for the mediation of hippocampal theta rhythm? Neuroscience 67:301–312

Tritsch NX, Granger AJ, Sabatini BL (2016) Mechanisms and functions of GABA co-release. Nat Rev Neurosci 17:139–145

Trottier S, Chotard C, Traiffort E, Unmehopa U, Fisser B, Swaab DF, Schwartz JC (2002) Co-localization of histamine with GABA but not with galanin in the human tuberomamillary nucleus. Brain Res 939:52–64

Tsunematsu T, Kilduff TS, Boyden ES, Takahashi S, Tominaga M, Yamanaka A (2011) Acute optogenetic silencing of orexin/hypocretin neurons induces slow-wave sleep in mice. J Neurosci 31:10529–10539

Tsunematsu T, Ueno T, Tabuchi S, Inutsuka A, Tanaka KF, Hasuwa H, Kilduff TS, Terao A, Yamanaka A (2014) Optogenetic manipulation of activity and temporally controlled cell-specific ablation reveal a role for MCH neurons in sleep/wake regulation. J Neurosci 34:6896–6909

Urbain N, Gervasoni D, Soulière F, Lobo L, Rentéro N, Windels F, Astier B, Savasta M, Fort P, Renaud B, Luppi PH, Chouvet G (2000) Unrelated course of subthalamic nucleus and globus pallidus neuronal activities across vigilance states in the rat. Eur J Neurosci 12:3361–3374

Urban DJ, Roth BL (2015) DREADDs (designer receptors exclusively activated by designer drugs): chemogenetic tools with therapeutic utility. Annu Rev Pharmacol Toxicol 55:399–417

Ursin R (2002) Serotonin and sleep. Sleep Med Rev 6:55–67

Valencia Garcia S, Libourel PA, Lazarus M, Grassi D, Luppi PH, Fort P (2017) Genetic inactivation of glutamate neurons in the rat sublaterodorsal tegmental nucleus recapitulates REM sleep behaviour disorder. Brain 140:414–428

Valencia Garcia S, Brischoux F, Clément O, Libourel PA, Arthaud S, Lazarus M, Luppi PH, Fort P (2018) Ventromedial medulla inhibitory neuron inactivation induces REM sleep without atonia and REM sleep behavior disorder. Nat Commun 9:504

Van Dort CJ, Zachs DP, Kenny JD, Zheng S, Goldblum RR, Gelwan NA, Ramos DM, Nolan MA, Wang K, Weng F-J, Lin Y, Wilson MA, Brown EN (2015) Optogenetic activation of cholinergic neurons in the PPT or LDT induces REM sleep. Proc Natl Acad Sci U S A 112:584–589

Vanni-Mercier G, Gigout S, Debilly G, Lin JS (2003) Waking selective neurons in the posterior hypothalamus and their response to histamine H3-receptor ligands: an electrophysiological study in freely moving cats. Behav Brain Res 144:227–241

Vardy E, Robinson JE, Li C, Olsen RHJ, DiBerto JF, Giguere PM, Sassano FM, Huang XP, Zhu H, Urban DJ, White KL, Rittiner JE, Crowley NA, Pleil KE, Mazzone CM, Mosier PD, Song J, Kash TL, Malanga CJ, Krashes MJ, Roth BL (2015) A new DREADD facilitates the multiplexed chemogenetic interrogation of behavior. Neuron 86:936–946

Varin C, Arthaud S, Salvert D, Gay N, Libourel PA, Luppi PH, Léger L, Fort P (2016) Sleep architecture and homeostasis in mice with partial ablation of melanin-concentrating hormone neurons. Behav Brain Res 298:100–110

Varin C, Luppi PH, Fort P (2018) Melanin-concentrating hormone-expressing neurons adjust slow-wave sleep dynamics to catalyze paradoxical (REM) sleep. Sleep 41(6). https://doi.org/10.1093/sleep/zsy068

Vazey EM, Aston-Jones G (2014) Designer receptor manipulations reveal a role of the locus coeruleus noradrenergic system in isoflurane general anesthesia. Proc Natl Acad Sci U S A 111:3859–3864

Venner A, Anaclet C, Broadhurst RY, Saper CB, Fuller PM (2016) A novel population of wake-promoting GABAergic neurons in the ventral lateral hypothalamus. Curr Biol 26:2137–2143

Vescia S, Mandile P, Montagnese P, Romano F, Cataldo G, Cotugno M, Giuditta A (1996) Baseline transition sleep and associated sleep episodes are related to the learning ability of rats. Physiol Behav 60:1513–1525

Vetrivelan R, Kong D, Ferrari LL, Madara JC, Bandaru S, Lowell BB (2016) Melanin-concentrating hormone neurons specifically promote rapid eye movement sleep in mice. Neuroscience 336:102–113

Vyazovskiy VV, Olcese U, Hanlon EC, Nir Y, Cirelli C, Tononi G (2011) Local sleep in awake rats. Nature 472:443–447

Watson CJ, Lydic R, Baghdoyan HA (2011) Sleep duration varies as a function of glutamate and GABA in rat pontine reticular formation. J Neurochem 118:571–580

Watson CJ, Baghdoyan HA, Lydic R (2012) Neuropharmacology of sleep and wakefulness: 2012 update. Sleep Med Clin 7:469–486

Weber F, Chung S, Beier KT, Xu M, Luo L, Dan Y (2015) Control of REM sleep by ventral medulla GABAergic neurons. Nature 526:435–438

Wieland S, Schindler S, Huber C, Kohr G, Oswald MJ, Kelsch W (2015) Phasic dopamine modifies sensory-driven output of striatal neurons through synaptic plasticity. J Neurosci 35:9946–9956

Williams RH, Jensen LT, Verkhratsky A, Fugger L, Burdakov D (2007) Control of hypothalamic orexin neurons by acid and CO2. Proc Natl Acad Sci U S A 104:10685–10690

Williams RH, Chee MJS, Kroeger D, Ferrari LL, Maratos-Flier E, Scammell TE, Arrigoni E (2014) Optogenetic-mediated release of histamine reveals distal and autoregulatory mechanisms for controlling arousal. J Neurosci 34:6023–6029

Wise A, Jupe SC, Rees S (2004) The identification of ligands at orphan G-protein coupled receptors. Annu Rev Pharmacol Toxicol 44:43–66

Xu M, Chung S, Zhang S, Zhong P, Ma C, Chang WC, Weissbourd B, Sakai N, Luo L, Nishino S, Dan Y (2015) Basal forebrain circuit for sleep-wake control. Nat Neurosci 18:1641–1647

Yamanaka A, Beuckmann CT, Willie JT, Hara J, Tsujino N, Mieda M, Tominaga M, Yagami KI, Sugiyama F, Goto K, Yanagisawa M, Sakurai T (2003) Hypothalamic orexin neurons regulate arousal according to energy balance in mice. Neuron 38:701–713

Yokota S, Kaur S, Vanderhorst VG, Saper CB, Chamberlin NL (2015) Respiratory-related outputs of glutamatergic, hypercapnia-responsive parabrachial neurons in mice. J Comp Neurol 523:907–920

Yu X, Ye Z, Houston CM, Zecharia AY, Ma Y, Zhang Z, Uygun DS, Parker S, Vyssotski AL, Yustos R, Franks NP, Brickley SG, Wisden W (2015) Wakefulness is governed by GABA and histamine cotransmission. Neuron 87:164–178

Yu X, Franks NP, Wisden W (2018) Sleep and sedative states induced by targeting the histamine and noradrenergic systems. Front Neural Circuits 12:4

Yuan XS, Wang L, Dong H, Qu WM, Yang SR, Cherasse Y, Lazarus M, Schiffmann SN, De Kerchove D'Exaerde A, Li RX, Huang ZL (2017) Striatal adenosine A2Areceptor neurons control active-period sleep via parvalbumin neurons in external globus pallidus. Elife 6:e29055

Zhang Z, Ferretti V, Güntan I, Moro A, Steinberg EA, Ye Z, Zecharia AY, Yu X, Vyssotski AL, Brickley SG, Yustos R, Pillidge ZE, Harding EC, Wisden W, Franks NP (2015) Neuronal ensembles sufficient for recovery sleep and the sedative actions of α2 adrenergic agonists. Nat Neurosci 18:553–561

Zhu H, Pleil KE, Urban DJ, Moy SS, Kash TL, Roth BL (2014) Chemogenetic inactivation of ventral hippocampal glutamatergic neurons disrupts consolidation of contextual fear memory. Neuropsychopharmacology 39:1880–1892