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A Decade of Orexin/Hypocretin and Addiction: Where Are We Now?

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Abstract

One decade ago, our laboratory provided the first direct evidence linking orexin/hypocretin signaling with drug seeking by showing that activation of these neurons promotes conditioned morphine-seeking behavior. In the years since, contributions from many investigators have revealed roles for orexins in addiction for all drugs of abuse tested, but only under select circumstances. We recently proposed that orexins play a fundamentally unified role in coordinating “motivational activation” under numerous behavioral conditions, and here we unpack this hypothesis as it applies to drug addiction. We describe evidence collected over the past 10 years that elaborates the role of orexin in drug seeking under circumstances where high levels of effort are required to obtain the drug, or when motivation for drug reward is augmented by the presence of external stimuli like drug-associated cues/contexts or stressors. Evidence from studies using traditional self-administration and reinstatement models, as well as behavioral economic analyses of drug demand elasticity, clearly delineates a role for orexin in modulating motivational, rather than the primary reinforcing aspects of drug reward. We also discuss the anatomical interconnectedness of the orexin system with wider motivation and reward circuits, with a particular focus on how orexin modulates prefrontal and other glutamatergic inputs onto ventral tegmental area dopamine neurons. Last, we look ahead to the next decade of the research in this area, highlighting the recent FDA approval of the dual orexin receptor antagonist suvorexant (Belsomra[®]) for the treatment of insomnia as a promising sign of the potential clinical utility of orexin-based therapies for the treatment of addiction.

Keywords

Addiction; Alcohol; Behavioral economics; Cocaine; Dopamine; Drugs of abuse; Glutamate; Heroin; Hypocretin; Motivation; Orexin; Reward; VTA

1 Introduction

Orexin peptides A and B (also called hypocretin 1 and 2) are produced by a limited number of neurons within the hypothalamus, ranging mediolaterally from the dorsomedial hypothalamic nucleus (DMH) through the perifornical area (PFA) into the lateral hypothalamus (LH) proper [1, 2]. Orexins signal via two G-protein-coupled receptors (orexin 1 and 2 receptors; Ox1R and Ox2R) that are distributed throughout the central nervous system [1, 2]. Orexin A has equal affinity for Ox1R and Ox2R, whereas orexin-B preferentially binds Ox2R [2]. Consistent with their anatomical position within the lateral hypothalamus, orexin neurons were originally identified as playing a key role in feeding behavior, with exogenous application of orexins shown to increase food intake [2, 3], and this effect being blocked by antagonism of orexin receptors [4]. This role for orexins in appetitive behavior has since been extended to a range of reward-related processes, including drug abuse and addiction [5–12].

Indirect evidence for a role for orexins in addiction came from a series of interesting clinical observations of patients suffering from narcolepsy with cataplexy, a neurological disorder associated with frequent sleep/wake transitions resulting from an almost complete loss of neurons producing orexin [13, 14]. A number of clinical case studies reported that narcoleptic patients rarely develop stimulant abuse, despite long-term treatment with stimulant medications including amphetamines [15, 16]. Consistent with this, Georgescu et al. [17] reported that orexin cells are activated (as seen with the immediate early gene product, Fos) after chronic morphine and withdrawal, and orexin knockout mice develop attenuated physical signs of morphine dependence compared to wild type controls.

In 2005, our laboratory directly implicated orexin signaling in drug-seeking behavior by showing that activation of LH orexin neurons is strongly correlated with conditioned preference for environmental contexts associated with cocaine or morphine [18] (Fig. 1). This study also showed that local chemical stimulation of LH orexin neurons reinstates extinguished drug seeking, and that this effect is blocked by systemic administration of an Ox1R antagonist (Fig. 1). Soon after, Boutrel and colleagues reported that central infusions of orexin peptide reinstate extinguished cocaine seeking and systemic injections of the Ox1R antagonist block cocaine seeking elicited by stress [19]. Together, these papers prompted a surge in preclinical studies examining the involvement of the orexins in various animal models of addiction, with >200 journal articles on orexin/hypocretin and addiction now published. As this number indicates, significant progress has been made in the last decade in understanding the specific roles for orexins in drug seeking and how they interact with the brain reward circuitry to drive these processes.

We recently articulated a hypothesis that the orexin system is preferentially engaged by situations of high motivational relevance, such as during physiological need states, exposure

to threats, or reward opportunities [11]. We suggest that orexin neurons translate this “motivational activation” into organized suites of psychological and physiological processes that support adaptive behaviors (Fig. 2). With respect to addiction behaviors, we and others have argued that orexin neurons are not involved in modulating the primary reinforcing effects of drugs of abuse per se, but are critical to regulating motivated responding for these drugs [7, 11, 20]. Key to this is the observation that motivation for drug reward can be enhanced by various external stimuli, including drug-associated stimuli and stress, and that this occurs in an orexin-dependent manner [21].

Here, we seek to synthesize the findings of the past 10 years that support the “motivational activation” hypothesis as it relates to drug addiction. We present evidence from studies using a range of behavioral models of addiction that highlight a motivational role for orexin in drug seeking. Next, we provide an overview of how orexin neurons interact with motivational and reward circuits to drive these processes, with a particular emphasis on how orexin neurons modulate the efficacy of glutamatergic inputs onto VTA neurons. We also discuss heterogeneity in orexin cell subpopulations, and the anatomical and functional characteristics that may contribute to the diversity of orexin neuron function. Finally, we seek to identify key gaps in our knowledge regarding orexin function in addiction that will be important to address in the coming decade of research in this field. Throughout this article, we focus primarily on psychostimulant drugs, as the role of orexin in alcohol use and abuse is covered elsewhere in this volume [22].

2 Orexin Signaling and Self-Administration Behavior

2.1 Traditional Self-Administration Models

Studies investigating the role of orexin in drug reward have frequently utilized the self-administration model, which allows investigators to examine responding for drug under schedules of reinforcement that differ in terms of the amount of effort required to obtain the drug. Under low fixed-ratio (FR) schedules of reinforcement (e.g., FR1, FR3), animals can easily maintain a preferred blood level of drug [23–25] and responding under these schedules allows insight into the primary reinforcing properties of the drug. Systemic administration of the selective Ox1R antagonist SB-334867 has no effect on cocaine or amphetamine self-administration under FR1 or FR3 schedules of reinforcement [19, 26–28], suggesting limited involvement of the orexin system in modulating the hedonic properties of psychostimulants.

Instead, orexin signaling is required for psychostimulant self-administration when higher levels of effort are required to obtain the drug. Systemic administration of SB-334867, as well as genetic deletion of Ox1R receptors, greatly reduces responding for cocaine on an FR5 schedule of reinforcement [29]. Similarly, under a progressive ratio (PR) schedule of reinforcement, where animals must expend exponentially increasing amounts of effort to obtain each subsequent drug infusion, systemic or intra-VTA injections of SB-334867 reduce the maximal effort the animal is willing to exert (as indicated by reduced breakpoints) [20]. Further, when motivation for cocaine is increased by intermittently restricting access to the drug, intra-VTA infusions of the orexin-A peptide increase, whereas intra-VTA infusions SB-334867 decrease, cocaine self-administration [26, 30]. These

findings are consistent with our recent results using behavioral economic procedures (described in Section 4) [21]. Importantly, these effects appear to be mediated primarily by the Ox1R, as selective Ox2R antagonists have no effect on highly motivated responding for cocaine [31].

2.2 Intracranial Self-Stimulation Models

Findings from self-administration studies are closely mirrored in studies that utilize the intracranial self-stimulation (ICSS) paradigm. In this paradigm, instead of responding for a drug reward, animals are required to make an operant response (generally turning a wheel) to deliver a rewarding electrical current into LH. When animals are trained to respond for LH stimulation under a continuous reinforcement schedule that requires low levels of effort (FR1) to obtain rewarding stimulation, SB-334867 has no effect on stimulation reward threshold, nor does it affect the ability of cocaine to reduce the minimum level of electrical current necessary to support self-stimulation [32]. In contrast however, the effects of cocaine on ICSS threshold are blocked by systemic SB-334867 when animals are trained to respond for ICSS under a discrete trial current-threshold procedure, which requires greater degrees of effort and motivation to obtain ICSS [29].

2.3 Conclusions

In summary, data from self-administration and ICSS studies point to a unique role for orexin in driving responding for psychostimulant reward under conditions of enhanced motivation. Orexin is required for psychostimulant self-administration behavior or ICSS responding only under schedules that require high amounts of effort to earn drug reward (FR5, PR). This is further supported by studies investigating orexin signaling in economic demand for psychostimulants (discussed in Section 4). In contrast, orexin is not necessary for the primary reinforcing effects of cocaine or amphetamine, or ICSS. Consistent with a more global role for orexin in modulating motivated behavior, SB-334867 also attenuates high-effort (FR5, PR) responding for other drugs of abuse, including alcohol [33–35], nicotine [36], and heroin [37], as well as high fat chocolate food (but not regular food or sucrose; [20, 33]). Unlike cocaine however, SB-334867 also affects low-effort (FR1 and FR3) responding for alcohol, nicotine, and heroin, indicating that there may be a role for orexin in mediating hedonic properties of some drugs [33, 36–38].

3 Orexin and Drug Seeking Elicited by External Stimuli

Environmental cues that are associated with drug use evoke motivational states that drive drug-seeking behavior, even in the absence of the drug. These cues engage the orexin system, which in turn acts to translate cue-induced motivation into appetitive actions. Studies investigating these processes utilize a variety of behavioral models, including conditioned place preference (CPP), operant reinstatement, and conditioned reinforcement paradigms. Exposure to stress is another potent stimulus known to drive drug seeking. Below, we discuss evidence from each of these paradigms that point to a central role for the orexin system in driving externally motivated drug-seeking behavior.

3.1 Orexin and Conditioned Place Preference

Harris et al. showed that activation of lateral (but not medial) hypothalamic orexin neurons is strongly correlated with CPP for cocaine and morphine [18] (Fig. 1). This study also reported that local chemical stimulation of LH orexin neurons reinstates extinguished CPP behavior, and that this effect was blocked by systemic administration of the Ox1R antagonist, SB-334867. Subsequent studies showed that systemic SB-334867 blocks the expression of CPP for amphetamine [27], and the dual OxR1 + OxR2 antagonist almorexant prevents the expression of cocaine CPP [39]. Expression of cocaine CPP depends on inputs to orexin neurons from the rostral lateral septum (LSr): Fos expression in LH-projecting LSr neurons is positively correlated with CPP, and inhibiting LSr cells prevents expression of CPP, and of Fos in LH orexin neurons [40]. Together, these findings are consistent with the notion that orexin neurons are engaged by stimuli (or environments) of high motivational relevance, and that this motivational activation is critical for the expression of reward-seeking behavior [11, 41].

3.2 Reinstatement Behavior

The orexin system is critically involved in the process of translating cue-induced motivation into appetitive actions. These processes are often probed experimentally using the reinstatement model of drug seeking whereby environmental cues paired with drug during self-administration are used to reinstate drug seeking following a period of abstinence or extinction training [12, 42]. Drug-associated cues are typically classified as either: (1) discrete; such as light or tone cues that are paired with cocaine infusions; (2) discriminative; whereby drug delivery is response-contingent but stimuli (lights, tones) predict drug availability (i.e., are not-response contingent); or (3) contextual; where animals are trained to self-administer and extinguish in two separate contexts with distinguishable compound cues (light, sound, olfactory or tactile cues), and reinstatement behavior is assessed by returning the animal to the original context where they learned to self-administer.

Systemic administration of SB-334867 dose-dependently blocks reinstatement of extinguished cocaine seeking elicited by discrete [21, 28, 43], discriminative [44], or contextual cues in rats [45] (Fig. 3). Similarly, systemic SB-334867 attenuates reinstatement of cocaine seeking when animals are returned to the self-administration chamber after either 1 day or 2 weeks of abstinence [45] (Fig. 3). These effects are driven by signaling at Ox1R, as pretreatment with the Ox2R antagonist 4PT had no effect on cue-induced cocaine seeking [27]. SB-334867 is also effective at blocking reinstatement of alcohol [34, 35, 46, 47], nicotine [48], and remifentanyl [49] seeking, and there is evidence that Ox2R antagonists block reinstatement of nicotine seeking [50], but not cue-induced alcohol seeking [51].

3.3 Conditioned Reinforcement

A model related to cue-induced drug seeking is the conditioned reinforcement (CR) paradigm, whereby animals learn to perform a novel response (e.g., nose poke) to receive a cue that was previously associated with drug delivery via another response (e.g., lever press) [52, 53]. Rats that were treated systemically with SB-334867 over 5 consecutive days of FR1 cocaine self-administration training showed attenuated responding for the cocaine-associated cue in a subsequent CR test [27]. This finding points to a role for orexin in

ascribing motivational significance to discrete cues during self-administration training. In contrast however, Smith et al. [28] reported that SB-334867 administered systemically prior to a single cocaine-cue conditioning session had no effect on the ability of those cues to elicit reinstatement of cocaine seeking following extinction training. The reasons for the discrepancy in these two sets of findings are unclear, but may be related to differences in motivational significance acquired by the cue over a single versus repeated conditioning trials, or to different roles for orexin in operant vs Pavlovian conditioning. Importantly, treatment with SB-334867 prior to the CR test also attenuates responding [27], which is consistent with findings outlined above relating to cue-induced reinstatement, and supports a role for orexin in cue-driven motivated responding.

3.4 Stress

Orexin neurons are well positioned to modulate the behavioral and physiological responses to stress via bidirectional connections with various stress-relevant regions including bed nucleus of the stria terminalis (BNST), central amygdala (CeA), paraventricular nucleus of the hypothalamus (PVN), and medial prefrontal cortex (mPFC; for an overview, see James et al. [54]). Indeed, orexin neurons are activated by various stressors [8, 55–58] and optogenetic stimulation of orexin neurons reduces time spent in the interaction zone in a social interaction test and increases freezing in an open field test [59, 60]. Further, orexin receptor antagonists reduce anxiety behavior, and attenuate neuroendocrine responses to stress [48, 55, 61, 62]. These findings are consistent with a global role for orexin involvement in situations of high motivational relevance and translating this into adaptive behavior [7, 11, 20].

Drug-seeking behavior is strongly influenced by stress. Extinguished drug seeking can be reinstated by exposure to a range of stressors, including brief (15 min) intermittent, unpredictable electrical footshock [63, 64], food deprivation [65], or a range of pharmacological stressors, including the anxiogenic drug yohimbine [66, 67]. Systemic administration of SB-334867 reduces reinstatement of cocaine seeking elicited by yohimbine in both male and female rats [43]. Further, reinstatement of cocaine seeking elicited by intracerebroventricular (ICV) infusions of orexin-A is blocked by systemic administration of a non-selective CRF receptor antagonist [19]. The BNST appears to be an important brain region for these processes, as SB-334867 blocks yohimbine-induced changes in excitatory transmission in dorsal anterolateral BNST that are associated with the reinstatement of cocaine seeking, and these changes are absent in prepro-orexin knockout mice [68]. Orexin may also act at the nucleus accumbens shell (NAcSh) to mediate stress-induced drug seeking, as local infusions of either SB-334867 or TCS-OX2-29 block footshock-induced reinstatement of morphine seeking [69]. In contrast, the VTA and dentate gyrus of the hippocampus are unlikely sites of action, as local infusions of SB-334867 into these sites do not affect stress-induced seeking of cocaine or morphine, respectively [70, 71].

3.5 Conclusions

In summary, orexin signaling is necessary for motivated psychostimulant seeking elicited by external triggers like discrete drug-associated cues, contexts associated with prior drug use, or stress. Such stimuli are known to elicit craving and relapse behavior in human addicts

[72–77]. This may reflect a role for orexin in conditioned responding for highly salient natural rewards, including certain foods and sex [20, 78, 79]. The orexin system may therefore offer a potential target for pharmacotherapies designed to reduce the risk of relapse during abstinence [10, 80, 81]. We propose that by decreasing OX1R signaling, and thereby attenuating responses of VTA dopamine neurons to cues associated with these rewards (discussed in Section 5.2.1), it may be possible to preferentially attenuate exaggerated craving responses without greatly affecting baseline reward processing. Indeed, many preclinical studies now suggest that natural reward seeking is unaffected following treatment with orexin receptor antagonists at doses that block drug-seeking behavior [9, 33, 44, 46].

4 Orexin and Behavioral Economics

4.1 Behavioral Economics Models

Behavioral-economic (BE) analyses of demand elasticity for drug and other rewards offer a sophisticated means by which to examine separately both reinforcing properties of a drug, and motivation to obtain and consume it. In a typical BE experiment, consumption of drug is plotted as a function of drug price, and both humans and animals are similarly responsive to increased drug price (in humans, monetary cost; in rats, lever presses). In rats, we employ a within-session BE paradigm [82, 83], and apply an exponential demand equation to generate estimates of preferred cocaine intake at zero cost (Q_0 , quantified by where computed demand curves intercept the ordinate) [84]. As such, Q_0 can be considered a measure of hedonic set point for a drug, or the consumption of a drug under 'free' (no cost) conditions. In addition, the slope of the demand curve conveys demand elasticity, or the sensitivity of drug seeking to increases in price. This elasticity parameter, termed alpha, is therefore an index of the animal's motivation to obtain drug, or to defend preferred blood levels of drug. Importantly, Q_0 and alpha are entirely orthogonal in cocaine demand tests, meaning that each parameter can be examined concurrently and independently [82, 85].

As described above, orexin is specifically involved in modulating motivated responding, as well as drug seeking elicited by external stimuli. A recent study in our laboratory used BE demand curve analysis to investigate these processes directly [21]. Different groups of rats were trained to self-administer cocaine with and without drug-paired discrete stimuli (light + tone), and demand curves for both groups of rats were calculated after pretreatment with SB-334867 or vehicle. Motivation (alpha) for cocaine was significantly higher when cocaine-associated cues were present and systemic administration of SB-334867 decreased motivation (increased alpha values) only in the presence of these cocaine-associated cues (Fig. 4). Further, SB-334867 had no effect on the hedonic set point (Q_0) for cocaine, regardless of whether cocaine-associated cues were present. These findings are highly consistent with a previous study using a similar BE thresholding procedure to demonstrate that SB-334867 does not affect cocaine consumption, but reduces the maximal number of responses that rats were willing to make to maintain self-administration (P_{Max}) [26].

The observation that cocaine-paired discrete stimuli increased motivation for cocaine reward is consistent with previous findings that drug-paired stimuli take on motivational properties that drive responding independently of the reinforcing effects of the cocaine itself [86]. Interestingly, this cue-driven enhancement of motivation for cocaine was blocked by

systemic SB-334867, indicating that signaling at Ox1Rs increases the reinforcing efficacy of cocaine-associated cues. These findings complement those outlined above, showing an important role for orexin signaling in stimulus-driven drug seeking. In contrast, blockade of orexin signaling had no effect on “free” consumption of cocaine (Q_0). Together, these findings are strongly consistent with the “motivational activation” hypothesis of orexin function [11].

5 Interaction Between Orexin Neurons and Brain Reward Circuitry

Orexin signaling is crucial for highly motivated and conditioned drug seeking, but how do orexin neurons interact with wider reward circuits in the brain to modulate motivation? Examination of the afferent and efferent projection patterns of orexin neurons reveals that these cells are highly interconnected with key motivational and reward regions. The topography of the orexin system is described in detail elsewhere [87, 88], as well as in other chapters of this volume. Here, we summarize studies that have provided anatomical or functional evidence of orexin-related circuits in psychostimulant-seeking behavior. In particular, we focus on orexin projections to the midbrain dopamine (DA) system, and how orexin modulates glutamatergic inputs to VTA, including those that arise from the prefrontal cortex.

5.1 Orexin Afferents

The first comprehensive study of inputs to orexin neurons was carried out by Sakurai and colleagues [89], using the retrograde tracer, tetanus toxin, that was genetically encoded to specifically reveal inputs to orexin neurons. Using this approach, the authors identified strong projections from various reward-related regions, including medial prefrontal cortex (mPFC), BNST, basal forebrain, basolateral and medial amygdala, arcuate nucleus, PVN, and median raphe nucleus (MRN). A subsequent study by Yoshida et al. [90] used the retrograde tracer cholera toxin B subunit (CTb) to identify afferents to LH and DMH orexin fields, and confirmed retrogradely labeled inputs using anterograde tracers and identification of appositions with orexin neurons. Using this approach, these authors confirmed many of the inputs identified by Sakurai et al., as well as strong projections from NAc shell, dorsolateral septum, ventral pallidum, central amygdala, VTA, and ventromedial and anterior hypothalamus. For a summary of afferent projections to orexin neurons from reward-related structures, see Fig. 5.

To identify inputs to orexin neurons that are important for reward seeking, our laboratory examined Fos expression in various forebrain regions during the expression of cocaine CPP in animals that had previously received injections of the retrograde tracer CTb into the lateral or medial orexin fields [40]. This approach revealed that neurons in the rostral lateral septum and ventral BNST that project to the lateral orexin field are uniquely activated during cocaine CPP, and the magnitude of this activation was correlated with the degree of CPP expression. In a follow-up experiment, bilateral disconnection of the septum-LH orexin pathway attenuated the expression of cocaine CPP, confirming a functional role for this input in the expression of reward seeking [40].

Very few studies have otherwise been carried out examining the functional role of orexin afferents in reward seeking. One region of potential interest is the NAc shell, as this region projects strongly to the LH orexin area [90–92] and inactivation of this region induces Fos expression in the LH orexin field and promotes alcoholic beer seeking [93] and feeding behavior [94]. Another study, while not identifying the source of inputs, showed that excitatory drive from glutamatergic neurons onto orexin neurons is increased following cocaine exposure [95]. Given the apparent role for the orexin system in driving motivational behavior, future studies may benefit from focusing on inputs from regions involved in motivational processes, including prefrontal cortex and striatopallidal structures.

5.2 Orexin Efferents

5.2.1 Ventral Tegmental Area—Neurons in the VTA, along with the neighboring substantia nigra pars compacta, are major sources of DA in the brain [96]. VTA DA neurons mediate the effects of drugs of abuse, including psychostimulants, and play a key role in modulating motivational processes of drug-seeking behavior [6, 97–99]. VTA neurons express both Ox1R and Ox2R, although there are a limited number of orexin-containing synapses in this region, suggesting that the majority of orexin input to VTA may be nonsynaptic *en passant* fibers or non-synapsing terminals [100]. Regardless, orexin-A directly depolarizes VTA DA neurons [101] and there is considerable evidence that orexin facilitates glutamatergic inputs to modulate VTA activity and reward seeking.

A growing literature points to the VTA as a key site of orexin signaling in motivated reward seeking. Local infusions of orexin A into VTA increase cocaine self-administration under a PR, but not an FR1, schedule of reinforcement [30]. Similarly, local SB-334867 injections reduce PR breakpoints and reduce effort expended in a BE task, but have no effect on FR1 responding for cocaine [26]. Intra-VTA infusions of orexin-A also elicit reinstatement of extinguished cocaine seeking and morphine CPP [18, 71], whereas VTA injection of SB-334867 blocks both discrete and discriminative cue-induced reinstatement of cocaine seeking [102, 103]. Similar findings have been reported for cue-induced reinstatement of ethanol seeking [104]. Orexin signaling in VTA increases DA release in forebrain structures like NAc, which is known to be important for reward seeking behavior [105–112]. Infusions of orexin-A into VTA increase DA release in prefrontal cortex and ventral striatum [30, 113], whereas infusions of SB-334867 reduce cocaine-induced DA in NAc [71], showing clear modulation of VTA DA neurons by orexin inputs.

VTA DA neuron firing is regulated by glutamate inputs [114–117], and orexin plays an important role in modulating these. Borgland et al. first demonstrated that orexin modulates glutamatergic signaling in VTA DA neurons [118]. Bath application of orexin-A facilitates glutamatergic transmission in VTA DA neurons, initially via facilitation of NMDA signaling, and later, via facilitation of AMPA signaling – both via postsynaptic mechanisms. VTA Ox1R antagonism also blocks cocaine-induced LTP of excitatory DA afferents in vitro, and blocks locomotor sensitization to cocaine in vivo. Orexin-B also enhances glutamatergic inputs to VTA DA neurons, but does so via presynaptic as well as postsynaptic mechanisms [119].

We followed up these findings by demonstrating a functional interaction between glutamate and orexin in VTA mediation of conditioned cocaine seeking [103]. First, we showed that intra-VTA SB-334867 blocked cue-induced reinstatement of cocaine seeking, as did intra-VTA blockade of AMPA receptors with CNQX. In addition, blockade of VTA orexin in one cerebral hemisphere, with simultaneous blockade of AMPA signaling in the contralateral hemisphere, similarly blocked cue reinstatement, indicating necessity of concurrent orexin and AMPA signaling for cues to elicit cocaine seeking. Moreover, we showed that reductions in cocaine seeking by intra-VTA SB-334867 were reversed when we concurrently administered the AMPA allosteric modulator PEPA into VTA, to potentiate AMPA signaling. Together, these results show that by facilitating AMPA signaling in DA neurons, orexin in VTA potentiates both DA neuron activity and motivated behavioral responses to cocaine cues. Although orexin potentiates NMDA signaling in VTA neurons as well [118, 119], we did not see reductions in cue-induced cocaine seeking following intra-VTA blockade of NMDA receptors with AP-5 microinjections. VTA NMDA transmission is most important for acquisition of reward learning (presumably via induction of LTP; [117]), whereas AMPA may instead mediate expression of cocaine seeking elicited by previously learned cocaine cues [12, 103]. Therefore, orexin is likely capable of facilitating both reward learning, and expression of motivation elicited by prior learning, via facilitation of glutamate transmission in VTA.

The sources of glutamate inputs to VTA DA neurons that might be facilitated by orexin include hypothalamic, mid- and hind-brain nuclei, and/or the large projection from prelimbic mPFC [120, 121]. Although mPFC electrical stimulation usually elicits only weak enhancements of DA neuron firing in anesthetized animals [122, 123], we asked whether orexin application to VTA would increase the efficacy of mPFC stimulation-induced DA neuron activation [124]. Indeed, application of a low concentration of orexin-A that does not itself affect DA neuron firing robustly facilitated DA neuron firing elicited by prelimbic stimulation. This demonstrates that mPFC inputs to VTA DA neurons are facilitated by orexin-A in vivo. This said, we note that we did not see activation (as measured with Fos) in VTA afferent neurons from either mPFC or hypothalamic orexin neurons themselves during cue-induced cocaine seeking (compared to various control behaviors; [125]). Intriguingly, non-orexin-A expressing-, VTA-projecting neurons in the LH orexin field were instead activated during cued cocaine seeking.

5.2.2 Other Efferent Regions—Beyond the VTA, only a handful of regions have been investigated as potential targets of orexin signaling in drug seeking, and the large majority of these studies have been carried out in alcohol-seeking models. Perhaps the next most comprehensively studied orexin efferent region is the paraventricular thalamus (PVT), a midline thalamic structure that is more densely innervated by orexin-positive fibers than any other forebrain structure [88]. The PVT expresses both Ox1R and Ox2R in high abundance [87], and bath application of orexin peptide potently depolarizes PVT neurons [126]. Recent evidence has implicated the PVT as a key structure in brain reward circuitry, and a clear role has been shown for this structure in a range of drug-seeking paradigms [9, 81, 127–133]. Dayas and colleagues provided the first evidence of an LH orexin-PVT circuit in drug seeking, by showing that PVT neurons that are activated by drug-associated cues are closely

apposed by orexin-positive fibers [129]. More direct evidence has come from studies showing that infusions of orexin-A and -B increase ethanol drinking and reinstate extinguished cocaine-seeking behavior. These effects appear to be mediated primarily via Ox2Rs, as Ox2R (but not Ox1R) levels in PVT are increased following ethanol drinking, and local infusions of the Ox2R antagonist TCS-OX2-29, but not the OxR1 antagonist SB-334867, reduces alcohol drinking [128]. A recent study suggests that signaling at the OxR2 in the PVT is also important for psychostimulant seeking, as infusions of orexin A into the PVT reinstated cocaine seeking and this was blocked by co-administration of TCS-OX2-29 but not SB-334867 [134]. These findings are consistent with an earlier demonstration that infusions of SB-334867 into the PVT have no effect on reinstatement of cocaine seeking elicited by discriminative cues [102].

The NAc is another potentially important site of orexin action in drug seeking, as this region expresses moderate to high levels of Ox2R [87, 135] and both orexin-A and B alter activity of NAc neurons [136, 137]. The effects of intra-NAc infusions of orexin receptor agonists or antagonists have not been examined for psychostimulants, but local infusions of SB-334867 or TCS-29-029 in NAcSh prevent stress-induced reinstatement of morphine conditioned place preference [69], and injections of TCS-OX2-029 in NAcC reduced responding for ethanol [51]. Also, injections of orexin-A into the rostral half of medial NAcSh increase the hedonic “liking” for sucrose taste [138], as do orexin-A injections into the posterior half of the ventral pallidum [139].

The BNST may be another target for orexin signaling in drug reward, as infusions of orexin-A into this region enhance alcohol seeking [140]. Also, as described above, OxR1 signaling in BNST appears to play an important role in stress-induced drug seeking. More recently, stress-induced reinstatement of alcohol seeking was shown to be dependent on signaling at the Ox₂R in the nucleus incertus, a small pontine region [141]. In the cortex, infusions of SB-334867 into the prelimbic cortex attenuate cue-induced reinstatement of ethanol seeking [104], and local infusions of SB-334867 in the insular cortex reduce nicotine self-administration behavior [36]. These findings highlight potential targets for orexin signaling beyond the mesocorticolimbic DA system that may be important in modulating psychostimulant-seeking behavior. For a summary of regions shown to be important sites of orexin signaling in drug seeking, see Fig. 6.

6 Functional Heterogeneity of Orexin Neurons: A Medial/Lateral Dichotomy in Orexin Cell Function?

Orexin neurons participate in a wide variety of functions including, but not limited to arousal, stress, and appetitive motivation [11, 143, 144]. A question arises as to how the orexin system participates in such diverse, and even apparently contradictory functions (e.g., stress and reward). One possibility is that there are heterogeneous subpopulations of orexin neurons that selectively participate in specific behavioral or physiological functions. There are multiple potential sources of heterogeneity in the orexin system. One of the more prominent factors appears to be differences in circuit functions of orexin neurons located in different hypothalamic subregions. Our laboratory proposed that orexin neurons in LH

preferentially encode reward motivation whereas orexin neurons in DMH and PFA process arousal and stress [8]. This proposal was initially based on our demonstration that LH orexin neurons were Fos-activated by cues associated with food, morphine, and cocaine but not footshock, whereas PFA-DMH orexin neurons were preferentially activated by arousal/anxiety-inducing footshock stimulation [8]. Furthermore, chemical activation of LH, but not PFA-DMH, orexin neurons reinstated preference for morphine [18]. We also noted studies in which activation of LH, and not DMH, orexin neurons was associated with antipsychotic-induced weight gain [145] as well as increased feeding associated with NAcSh inhibition [94]. Conversely, PFA-DMH orexin neuron Fos activation was preferentially associated with wakefulness and arousal [145–147] as well as footshock, restraint, and cold stress [148, 149].

Since the publication of Harris and Aston-Jones [8], a number of studies have been performed investigating the activation of orexin neurons in a variety of appetitive and aversive contexts. In many cases, the distinction between lateral neurons encoding appetitive motivated behaviors and medial neurons encoding arousal or aversive motivated behaviors has been replicated. Work from our lab has shown preferential encoding of drug preference in LH orexin neurons [40, 150–152] and preferential signaling of arousal by PFA/DMH orexin neurons [153]. A number of other groups have also found preferential encoding of reward seeking by lateral orexin neurons. Context-induced reinstatement (ABA renewal) of beer seeking induced Fos activation of PFA and LH, but not DMH, orexin neurons, and the activity of LH orexin neurons (and not PFA/DMH) was correlated with strength of renewal [154]. Increased Fos activation of DMH and PFA, but not LH orexin neurons was observed following ABA renewal of cocaine seeking, though this increase was also seen in ABB controls in which animals were tested in their extinguished environment (B) instead of the conditioned environment (A) [91]. This is similar to the increased Fos observed in PFA and DMH (but not LH) orexin neurons during extinction from sucrose self-administration [78].

The increased reinstatement for cocaine seeking following ICV infusions of neuropeptide S depended primarily on LH orexin neurons [155]. Alcohol seeking induced by baclofen/muscimol infusions in the NAc induced Fos activation of PFA and LH, but not DMH, orexin neurons [93]. Morphine CPP preferentially activated LH, and not PFA or DMH, orexin neurons [156]. In a recent report, LH orexin neurons exhibited stronger firing in response to reward- vs. punishment-predicting cues [157], though the authors noted that LH orexin neurons also exhibited activity correlations with arousal in their previous studies [158].

Also supporting lateral vs. medial differences in orexin neuron function is the finding that medial orexin neurons preferentially encode arousing or aversive stimuli, events, and outcomes. Unpredictable chronic mild stress, a rodent model of depression, increased Fos expression in PFA/DMH orexin neurons, but not LH [159]. Naloxone-induced morphine withdrawal activates DMH and PFA, but not LH orexin neurons [160]. Anxiogenic doses of acute nicotine increased Fos in PFA/DMH but not LH, whereas cue-induced reinstatement of nicotine seeking increased Fos in PFA and LH but not DMH orexin neurons [48, 161]. DMH and PFA, but not LH neurons are activated by panic induced by sodium lactate, CO₂, caffeine, or the anxiogenic drug FG-7142 [62, 162–164]. Inflammation using formalin

injections into the hindpaw increased Fos in DMH and PFA, but not LH orexin neurons, and this activation was correlated with the extent of licking and grooming responses [165].

However, a medial/lateral dichotomy in orexin neuron function has not been observed in every study (see Tables 1 and 2). Stressful situations such as exposure to a brightly lit novel environment induced Fos activation of medial and lateral orexin neurons [172], as did a range of behaviors in mice, including progressive ratio responding for food reward [173]. In one study, restraint stress resulted in increased Fos in LH but not PFA/DMH orexin neurons [166]. Discriminative stimulus-driven reinstatement of alcohol seeking increased Fos activity in DMH, PFA, and LH orexin neurons [129]. Similarly, in a conditioned sucrose-seeking model, orexin neuron activation was observed across all orexin cell fields in food-restricted rats [78]. Cue-evoked feeding in sated animals produced preferential activation of PFA orexin neurons [174, 175], as did expectation of chocolate [176], while feeding induced by DAMGO infusions in the medial prefrontal cortex [177] or NAc [178] activated medial but not lateral orexin neurons. Finally, in a recent study from our laboratory, we found that the strength of context-induced reinstatement of ethanol seeking was correlated with the strength of Fos activation of LH and DMH (but not PFA) orexin neurons [151]. Together, these results indicate that the lateral vs. medial dichotomy in orexin neuron function may not always be as simple as encoding appetitive vs. aversive behaviors, respectively. The role of the PFA orexin neurons, which in some cases appears to associate more with appetitive and in other cases with aversive behaviors, needs to be investigated further.

As shown in Table 2, there may be a dichotomy whereby Pavlovian reward-associated behaviors (such as conditioned place preference) primarily engage LH and not PFA orexin neurons. In contrast, instrumental reward seeking, often performed in a reinstatement context in which the primary reinforcer is unavailable, recruits both LH and PFA neurons. One possible explanation for this observation is that instrumental reinstatement in the absence of a reward produces both motivation to acquire reward along with frustration in not receiving reward, thus engaging both LH and PFA (and sometimes DMH) orexin neurons. Another possible explanation may arise from the fact that instrumental, and not Pavlovian, reward-related behaviors require increased arousal and/or motor activity, in addition to motivation for reward. The simultaneous increase in arousal/motor output as well as reward motivation thus activates both LH and PFA orexin neurons to facilitate an active reward-seeking behavior, hypotheses presented in previous reports [8, 173]. These proposals should be directly tested in behavioral paradigms designed to isolate subcomponents of reward seeking, arousal, motor behavior, etc., in order to identify specific relationships to orexin neuron activity.

In addition, it will be important to consider different behavioral categories beyond simply appetitive vs. aversive, which may further dissect selective contributions of orexin neuron populations. Future work will also benefit from characterizing real-time activation of orexin neurons (as shown, for example, in [157, 158, 179]), direct modulation of orexin neuron populations [59, 180–183], and correlating strength of activation with specific behaviors [18, 151, 154].

Differential anatomical connectivity of neurons within orexin subfields may provide a basis for functional differences between lateral vs medial orexin cell groups. In support of this possibility, a number of groups reported that different brain areas innervate lateral vs. medial orexin fields [90, 184]. Although somewhat speculative, an overview of the regions that preferentially project to the lateral vs. medial orexin fields reflects regions that could be broadly defined as appetitive/motivational vs. arousal/anxiety categories, respectively. For example, projections from dorsal mPFC in rats (prelimbic and cingulate), which are frequently associated with expression of conditioned fear [185], target medial orexin fields, whereas ventral medial prefrontal cortex (infralimbic) and orbitofrontal cortex, which are commonly associated with extinction of fear and reward seeking [185], preferentially target lateral regions of the orexin field [184]. As discussed above, studies in mice using genetic tools for identifying neuronal populations specifically projecting to orexin neurons revealed a range of brain areas including those related to sleep/wake (e.g., basal forebrain, median raphe, and ventrolateral preoptic area) as well as motivation and emotion (e.g., infralimbic medial prefrontal cortex, amygdala, NAc, BNST, and lateral septum) [89]. In these studies, however, lateral vs. medial differences were not considered indicating that the interaction between orexin neuron anatomical location and afferents may be a useful research path.

There is also evidence for differential physiological properties of orexin neurons based on projection targets; this provides another possible basis for functional heterogeneity among orexin cell groups. As outlined above, orexin neurons signal at a number of target structures to regulate drug seeking, including VTA [20, 30, 71, 102, 103], PVT [128, 131, 132], and other reward and motivation-related targets [12, 186]. Early work revealed that lateral (LH/PFA) orexin neurons preferentially projected to VTA in rats [187], although orexin neuron projections to VTA in mice appear to originate from both medial and lateral subdivisions [188]. Only a small number of studies to date have explored the function of orexin neurons based on their projection targets [150, 189]. Work from our lab demonstrated that VTA-projecting, but not locus coeruleus-projecting, orexin neurons were activated during morphine conditioned place preference [152]. Interestingly, the same study demonstrated that VTA-projecting LH, but not PFA/DMH orexin neurons exhibited Fos activation correlated with CPP scores. These and other results indicate that selective projections of specific orexin neuron populations may serve as a mechanism for differentiating functional ensembles.

Orexin neurons co-express other neurotransmitter molecules, and this provides another possible basis for functional differences in orexin neuron populations [11]. Such co-expressed signaling molecules include classical neurotransmitters (eg. glutamate [190–192]) and neuropeptides (eg. dynorphin [193, 194]).

Finally, differences in physiological properties may also contribute to functional heterogeneity of orexin neuron subpopulations. Burdakov and colleagues have characterized two distinct populations of orexin neurons based on a combination of physiological (transient vs. persistent inhibitory responses to glucose administration *in vitro*) and anatomical (medial vs. lateral) location [195]. Additional studies may further delineate subpopulations of orexin neurons by endogenous physiological properties.

6.1 Summary

Different populations of orexin neurons may preferentially participate in specific functions within the greater context of motivational activation. Whether the primary differentiating factor is anatomical location within the medial/lateral axis of the hypothalamus, as our group proposed 10 years ago [8], has so far not been conclusively proven or dismissed. A likely future outcome will be the revelation that a confluence of factors – anatomical, hodological, physiological, and molecular – will differentiate orexin neurons into specific functional populations. Future integrative studies, using well-designed behavioral paradigms to isolate specific physiological or behavioral factors, will ultimately assist in understanding how the range of functionally distinct subpopulations of orexin neurons, and how these populations are segregated and how they interact, both with each other and with other hypothalamic neuronal populations.

7 Conclusions and Future Directions

The past decade has seen significant advancement of our understanding of the orexin system in reward and addiction. In drug-seeking paradigms, orexin neurons are preferentially engaged under circumstances where high levels of effort are required to obtain drug, or when motivation for drug is augmented by external stimuli such as drug-related cues or stress. These findings are consistent with what we believe constitutes a broader, fundamentally unified role for the orexin system in facilitating motivational states – both appetitive and aversive – and translating these states into behaviors that the organism uses to exploit a reward opportunity or respond to pressing threats – a process we termed “motivational activation” [11]. We suggest that this diverse, integrative role must be the result of heterogeneous functional connectivity with wider brain circuits that allow orexin neurons to modulate a range of behavioral and physiological outputs. Indeed, a priority of future studies must be to characterize this heterogeneity. As we discuss above, we believe that an important first step will be examining how different orexin subpopulations (for example, medial versus lateral) differ in terms of their precise afferent and efferent topography. Recent advances in cell-specific targeting approaches that allow for the selective regulation of orexin neurons and their afferents or efferents, using either optogenetics or chemogenetics (e.g., DREADDs) will allow these questions to be addressed at both an anatomical and functional level.

We note that the majority of studies of orexin in the context of drug abuse have been carried out in short-access models that may not fully recapitulate the behavioral features of addiction [196]. It is imperative that future studies examine how the orexin system is involved in the compulsive behavioral phenotypes that are precipitated by alternative models such long [196, 197] or intermittent [198] access models. Indeed, rats that are exposed to these paradigms exhibit behavioral characteristics reflective of increased motivational drive for drug, including escalated cocaine intake and compulsive drug use despite punishment. Currently, our laboratory is investigating how the orexin system may be involved in this motivational shift and whether this system can be targeted to reduce addiction-like behaviors.

In August 2014, the dual orexin receptor antagonist Suvorexant (Belsomra®) was approved by the US FDA for the treatment of insomnia. Clinical trials suggest that this drug is relatively well tolerated with few side effects reported at doses required to achieve sleep-promoting effects [199]. In addition, a number of other orexin receptor antagonist compounds are also currently at various stages of preclinical and clinical testing [10]. As such, there is significant interest in the potential use of orexin receptor antagonists for the treatment of other disorders associated with orexin dysfunction, including substance use disorders. Although we agree that these compounds may offer an exciting new approach to treating psychostimulant addiction – for which there are currently no effective pharmacotherapies – we also caution that our understanding of the orexin system in addiction is far from complete. This said, the rapid growth in our understanding of orexin in the last 10 years, including the introduction of the first orexin receptor antagonist for clinical use, bodes well for the next 10 years of research into this intriguing neuropeptide system.

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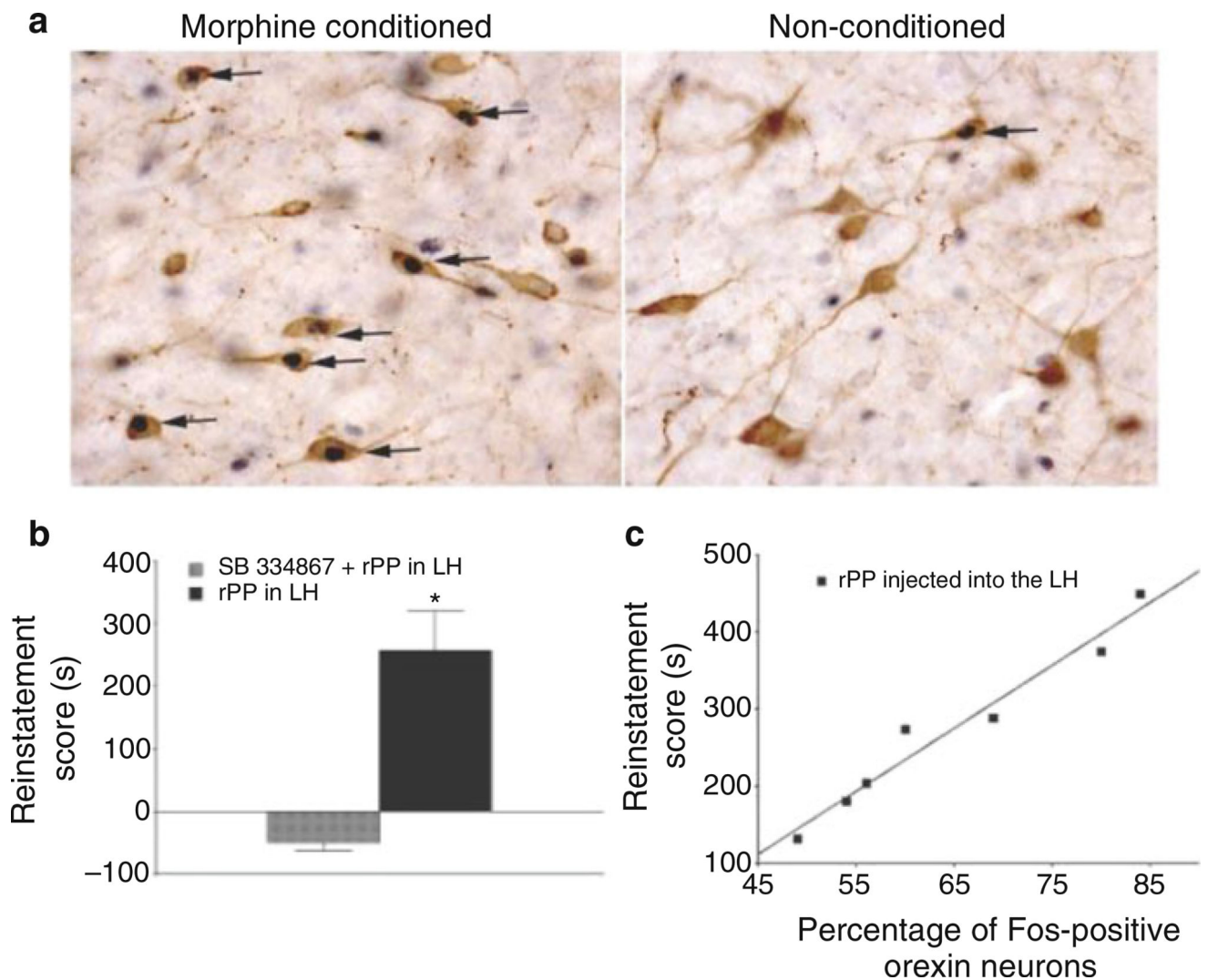
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**Fig. 1.**

First direct evidence that orexin signaling is involved in drug seeking behavior. (a) Using a two-chamber, non-biased, conditioned-place preference paradigm, animals were trained to associate one chamber with drug (morphine or cocaine) reward, whereas the other chamber was associated with no reward. Conditioned animals displayed reward-seeking by spending significantly more time in the reward-paired chamber. To determine if orexin neurons were stimulated during the expression of this preference, tissue was dual-labeled for both orexin and the immediate early gene protein Fos (a marker of neuronal stimulation). Only conditioned animals that exhibited a preference for the reward-paired chamber showed increased Fos expression in LH orexin cells. Similar percentages of orexin neurons in LH were activated following preference testing for morphine or cocaine. (b) Additional experiments sought to demonstrate that activation of orexin neurons could reinstate extinguished drug seeking. Animals were submitted to morphine conditioned place preference conditioning and then this behavior was extinguished by repeatedly exposing the rats to the chambers without morphine administration. To activate orexin neurons, animals received intra-LH infusions of the Y4 receptor agonist rPP (rat pancreatic polypeptide), as

orexin neurons express the Y4 receptor and activation of this receptor by rPP induces Fos in these neurons. Infusions of rPP into LH robustly reinstated an extinguished morphine place preference, and this effect was blocked by pretreatment with the selective orexin 1 receptor antagonist SB-334867. (c) rPP-induced Fos expression in orexin neurons was strongly correlated with reinstatement score. Figure adapted from Harris et al. [18]

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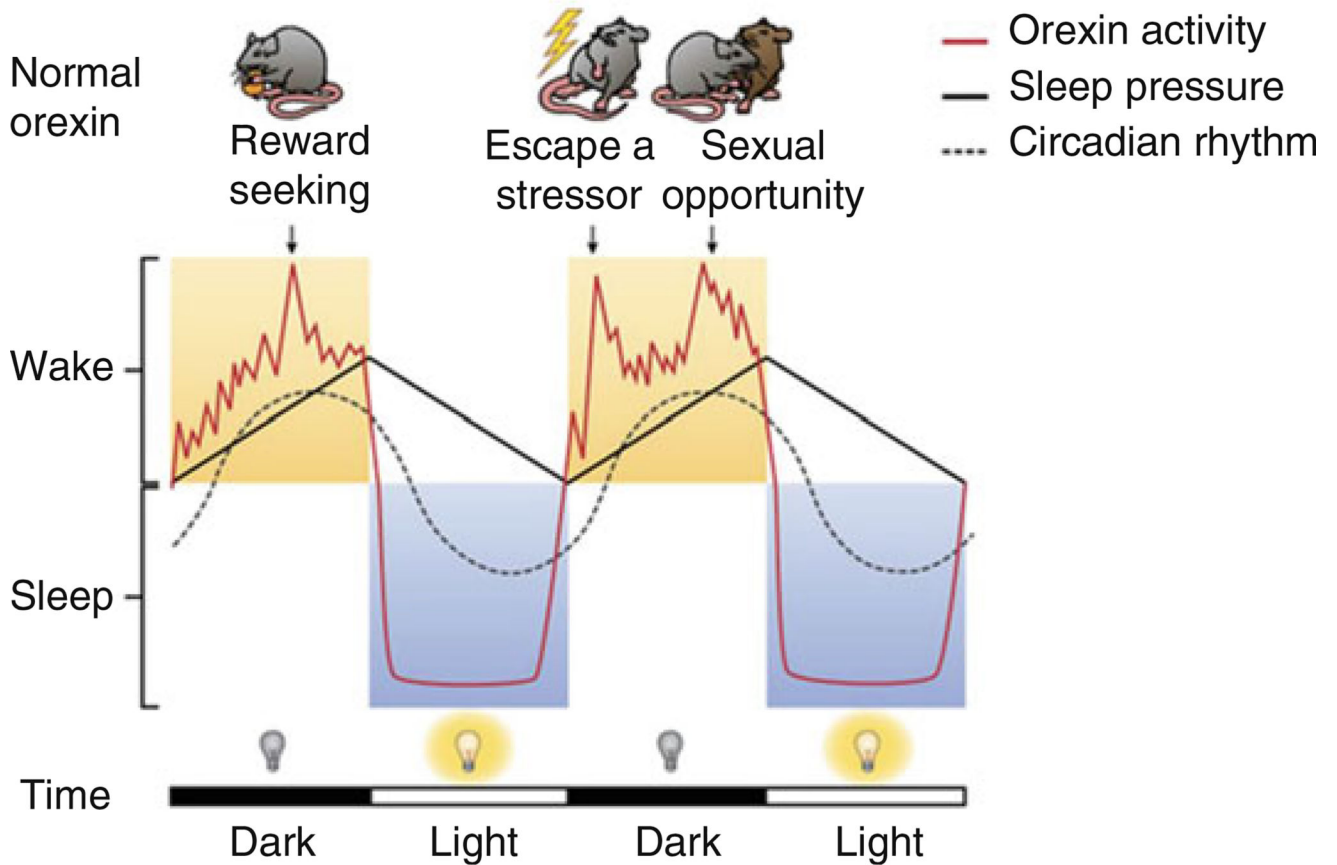


Fig. 2.

“Motivational activation”: a unifying hypothesis of orexin/hypocretin function. We propose that orexin neurons have a fundamentally unified role that spans a variety of processes, including arousal, reward seeking, homeostatic regulation, and stress, which we term “motivational activation.” Orexin neuron activity (*red line*) has a circadian-like pattern but also exhibits phasic bursts during waking as a function of motivational state and adaptive behavior, such as reward seeking. We hypothesize that both suprachiasmatic nucleus-dependent tonic excitation and phasic orexin neuron burst firing help to produce and maintain wakefulness – the latter as a consequence of increased motivation to engage adaptive challenges or opportunities. Adapted from Mahler et al. [11]

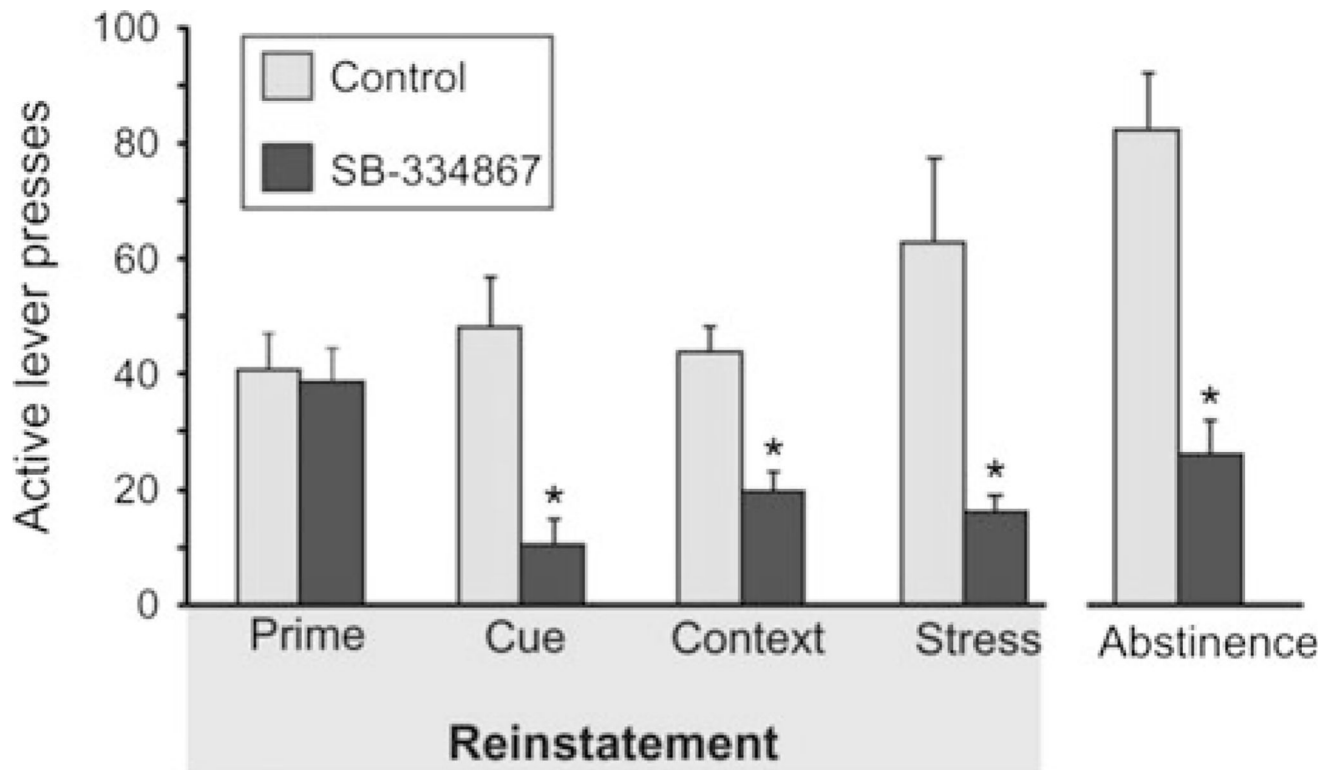


Fig. 3. Effects of SB-334867 treatment on reinstatement of cocaine seeking behavior. Systemic treatment with the orexin 1 receptor antagonist SB-334867 (30 mg/kg, i.p.) blocks reinstatement of cocaine seeking elicited by discrete cues, contextual cues, and stress. In contrast, SB-334867 has no effect on cocaine seeking elicited by a priming injection of cocaine. Figure adapted from Mahler et al. [12]

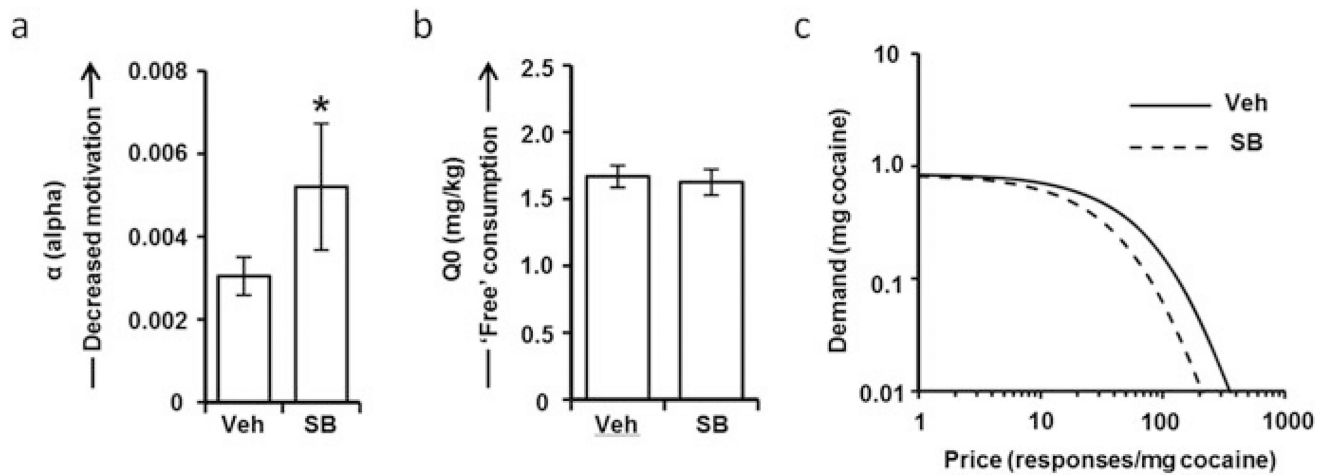


Fig. 4. Effects of SB-334867 treatment on economic demand for cocaine. **(a)** Pretreatment with SB-334867 increased cocaine demand elasticity (α), reflecting a decrease in motivation for cocaine. **(b)** SB-334867 had no effect on free cocaine consumption (Q_0), consistent with previous studies showing that the orexin system is not involved in mediating the hedonic properties of cocaine. **(c)** Demand curves representing increased sensitivity of demand to price following SB-334867 treatment. Figure adapted from Bentzley et al. [21]

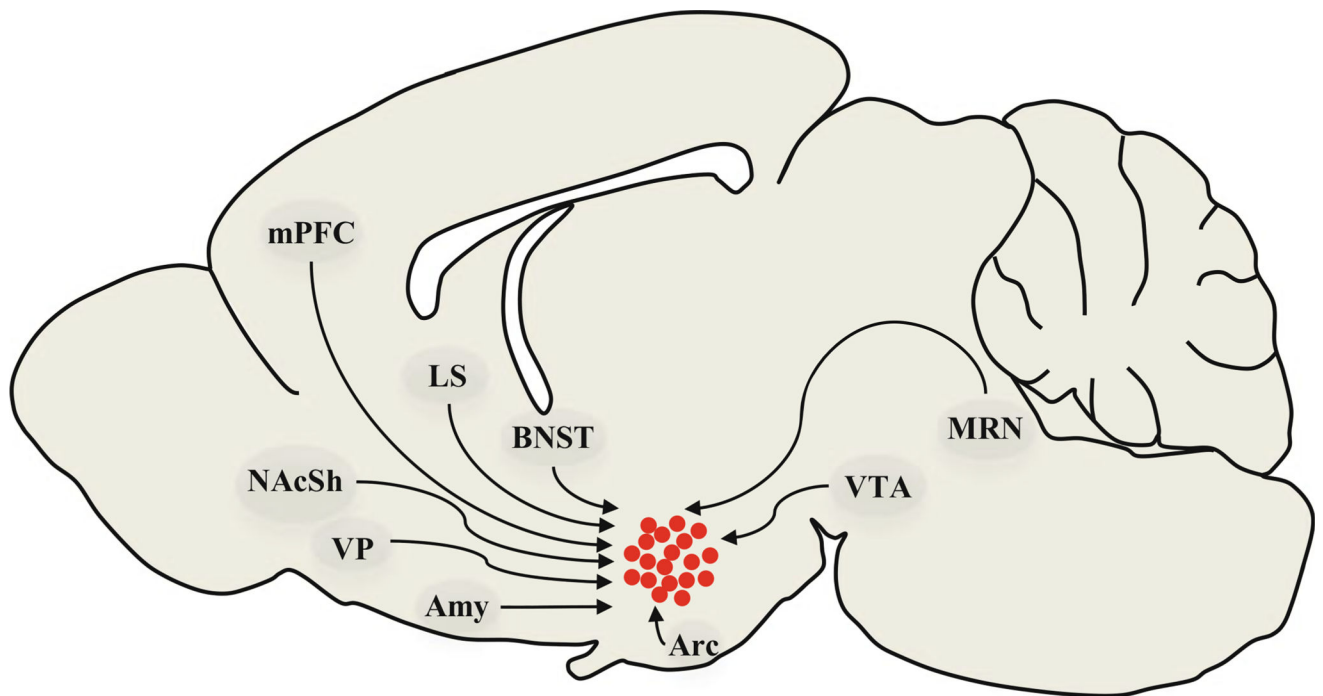


Fig. 5. Afferent projections to orexin neurons from reward-related structures. A mid-sagittal section of a rat brain illustrating loci involved in drug seeking that provide input onto orexin neurons. *Amy* amygdala (central, medial, and basolateral amygdala provide input to orexin neurons), *Arc* arcuate nucleus of the hypothalamus, *BNST* bed nucleus of the stria terminalis, *LS* lateral septum, *mPFC* medial prefrontal cortex (both prelimbic and infralimbic structures), *MRN* medial raphe nucleus, *NAcSh* nucleus accumbens shell, *VP* ventral pallidum, *VTA* ventral tegmental area

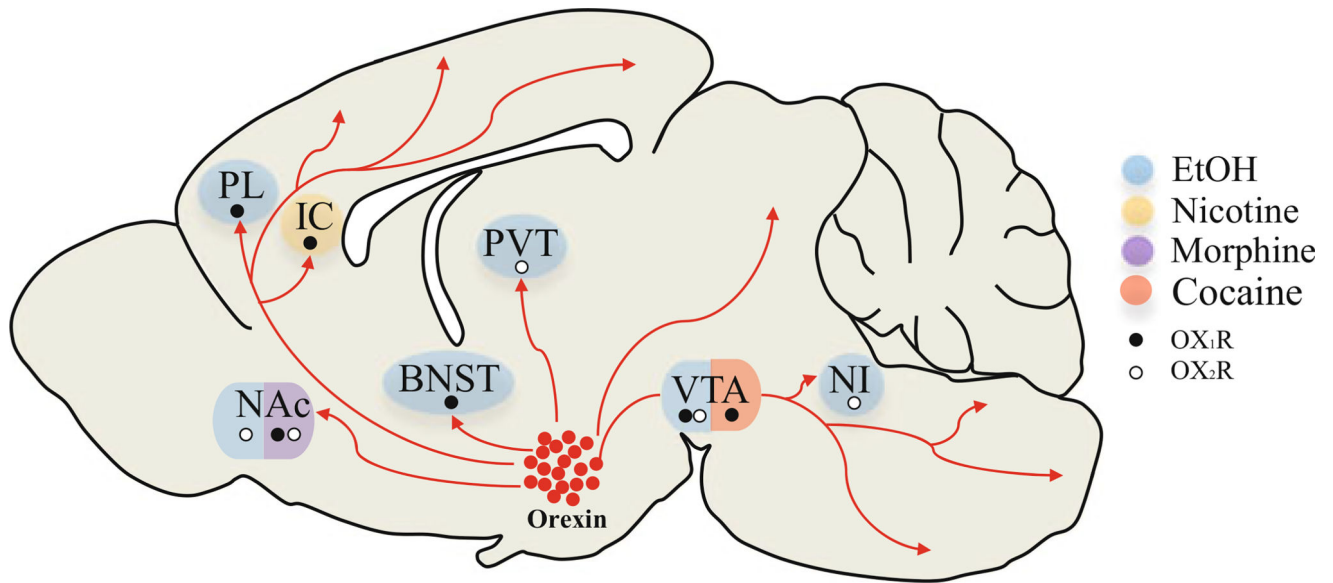


Fig. 6. Sites of orexin signaling in drug-seeking behavior. Orexin neurons in the lateral hypothalamus project to a number of reward structures involved in drug-seeking behavior. The ventral tegmental area (VTA) has been identified as an important site of orexin signaling in cocaine seeking behavior (*red*). Intra-VTA infusions of the orexin receptor 1 antagonist SB-334867 attenuate reinstatement of cocaine seeking elicited by discriminative stimuli, discrete stimuli, and stress [71, 102, 103]. Similarly, intra-VTA infusions of SB-334867 block cue-induced reinstatement of ethanol seeking [104], and infusions of the dual orexin receptor antagonist almorexant into the VTA reduce alcohol drinking [142]. Ethanol-seeking behaviors have been shown to be dependent on orexin signaling at other loci, including the prefrontal cortex (PL; [104]), nucleus accumbens (NAc; [51]), the anterior paraventricular thalamus (PVT; [128]), the bed nucleus of the stria terminalis [140], and the nucleus incertus (NI; [141]). Signaling at both the OX1R and OX2 in the NAc is important for stress-induced reinstatement of morphine conditioned place preference [69], and infusions of SB-334867 into the insular cortex (IC) reduce nicotine seeking behavior [36]. (PNAS)

Table 1
Summary of studies that have reported patterns of orexin neuron activation under conditions of stress or arousal

	Stimuli/paradigm	DMH orexin	PEA orexin	LH orexin	Reference
Stress	Acute amphetamine injection (1.5 mg/kg; i.p.)	↑ Fos ^a	↑ Fos ^a		Fadel et al. [145]
	Acute amphetamine injection (0.5 mg/kg; i.p.)	<i>b</i>	↑ Fos (correlated with wakefulness)	<i>b</i>	Estabrooke et al. [146]
	Acute caffeine injection (10, 30, 75 mg/kg; i.p.)	↑ Fos	↑ Fos	↑ Fos (10, 30 mg/kg dose)	Murphy et al. [147]
	Acute caffeine injection (50 mg/kg; i.p.)	↑ Fos ^a	↑ Fos ^a		Johnson et al. [163]
	Acute nicotine injection (0.8 mg/kg; s.c.)	↑ Fos ^a	↑ Fos ^a		Plaza-Zabala et al. [48]
	Footshock	↑ Fos	↑ Fos		Harris and Aston-Jones [8]
	Footshock	<i>b</i>	↑ Fos	<i>b</i>	Winsky-Sommerer et al. [149]
	Immobilization stress (30 min)	<i>b</i>	↑ Fos	<i>b</i>	Winsky-Sommerer et al. [149]
	Immobilization stress (20 min)	<i>c</i>	↑ Fos ^c	<i>c</i>	Sakamoto et al. [148]
	Immobilization stress (30 min; used to reinstate cocaine CPP)	<i>a</i>	<i>a</i>	↑ Fos	Tung et al. [166]
	Cold water stress	<i>b</i>	↑ Fos ^b	<i>b</i>	Sakamoto et al. [148]
	Carbon-dioxide stress	↑ Fos ^a	↑ Fos ^a		Sunaga et al. [164] and Johnson et al. [167]
	Sodium lactate injections	↑ Fos	↑ Fos		Johnson et al. [62]
	FG-7142 injections (7.5 mg/kg)	↑ Fos ^a	↑ Fos ^a		Johnson et al. [163]
Arousal	Hindpaw formalin injections	↑ Fos (correlated with pain response)	↑ Fos (correlated with pain response)		Campbell et al. [165]
	Naloxone-induced morphine withdrawal	↑ Fos	↑ Fos		Sharf et al. [160]
	Unpredictable chronic mild stress	↑ Fos ^a	↑ Fos ^a		Nollet et al. [159]
	Sleep deprivation	<i>b</i>	↑ Fos (correlated with wakefulness)	<i>b</i>	Estabrooke et al. [146]
	Active vs rest period	↑ Fos in active period	↑ Fos in active period (correlated with wakefulness)		Estabrooke et al. [146]
	Active vs rest period	↑ Fos in active period	↑ Fos in active period		Gompf and Aston-Jones [153]
	Running wheel confinement-induced resetting of circadian clock	↑ Fos	↑ Fos	↑ Fos	Webb et al. [168]
	Wakefulness induced by inhibition of basal forebrain	↑ Fos	↑ Fos	↑ Fos	Satoh et al. [111]

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Stimuli/paradigm	DMH orexin	PFA orexin	LH orexin	Reference
Acute modafinil injection (150 mg/kg; i.p.)	<i>b</i>	↑ Fos	<i>b</i>	Chemelli et al. [169]

In cases where cell is blank, no effect of the experimental manipulation was observed in this region

a Indicates that PFA and DMH regions were not quantified separately

b Indicates that this region was not examined

c Indicates that in this paper, subregions were not quantified separately in the original paper – subregional effects are estimated here based on original figure depicting Orx+/Fos+ cells across entire orexin field

Table 2

Summary of studies that have reported patterns of orexin neuron activation in Pavlovian vs. instrumental reward seeking paradigms

		Stimuli/paradigm	DMH orexin	PFA orexin	LH orexin	Reference	
Pavlovian	Opiates	Morphine CPP			↑ Fos (Fos correlated with CPP)	Harris and Aston-Jones [18], Harris et al. [150], and Lasheras et al. [156]	
		Morphine CPP			↑ Fos in VTA-projecting neurons (Fos correlated with CPP)	Richardson and Aston-Jones [152]	
	Cocaine	Cocaine CPP			↑ Fos (Fos correlated with CPP)	Harris and Aston-Jones [18]	
Instrumental	Cocaine	Cocaine CPP	^a	^a	↑ Fos in LS-projecting neurons (Fos in LH-projecting LS neurons correlated with CPP)	Sartor and Aston-Jones [40]	
		ABA renewal	↑ Fos (also seen in ABB)	↑ Fos (also been in ABB)		Hamlin et al. [91]	
		Cued-reinstatement (with NPS infusions)		↑ Fos	↑ Fos	Kallupi et al. [155]	
	Alcohol	Context-induced reinstatement	Fos correlated with seeking			Fos correlated with seeking	Moorman et al. [151]
		ABA renewal (beer)		↑ Fos	↑ Fos (Fos correlated with seeking)	↑ Fos (Fos correlated with seeking)	Hamlin et al. [154]
		DS+ reinstatement	↑ Fos	↑ Fos	↑ Fos	↑ Fos	Dayas et al. [129]
		EtOH-seeking elicited by inhibition of NAcSh		↑ Fos	↑ Fos	↑ Fos	Millan et al. [93]
	Nicotine	Homecage EtOH seeking				Fos correlated with seeking	Moorman et al. [151]
		Cue-induced reinstatement				↑ Fos	Plaza-Zabala [170]
		Methamphetamine self-administration				↑ Fos (not seen in meth-seeking rats)	Cornish et al. [171]

In case where cell is blank, no effect of the experimental manipulation was observed in this region

^aIndicates that PFA and DMH regions were not quantified separately