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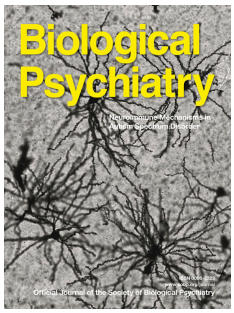

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Orexin reserve: A mechanistic framework for the role of orexins (hypocretins) in addiction

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Abstract

In 2014 we proposed that orexin signaling transforms motivationally relevant states into adaptive behavior directed toward exploiting an opportunity or managing a threat, a process we referred to as 'motivational activation'. Advancements in animal models since then have permitted higher-resolution measurement of motivational states; in particular, the behavioral economics approach for studying drug demand characterizes conditions that lead to the enhanced motivation that underlies addiction. This 'motivational plasticity' is paralleled by persistently increased orexin expression in a topographically-specific manner – a finding confirmed across species, including in humans. Normalization of orexin levels also reduces drug motivation in addiction models. These new advancements lead us to update our proposed framework for orexin function. We now propose that the capacity of orexin neurons to exhibit dynamic shifts in peptide production contributes to their role in adaptive motivational regulation, and that this is achieved via an orexin neuron 'reserve'. This reserve is normally bidirectionally recruited to permit motivational plasticity that promotes flexible, adaptive behavior. In pathological states such as addiction, however, we propose that the orexin system loses capacity to adaptively adjust peptide production, resulting in focused hypermotivation for drug driven by aberrantly and persistently high expression in the orexin reserve. This mechanistic framework has implications for understanding and treating several psychiatric disorders beyond addiction, particularly those characterized by motivational dysfunction.

The orexin system as a 'motivational activation' system

The brain orexin/hypocretin (referred to here simply as 'orexin') system originates solely from neurons in posterior hypothalamus but includes projections throughout the central nervous system [1]. Studies over the last 20 years since the orexin system was discovered [2, 3] have implicated these hypothalamic neurons in several behaviors. Originally, this system was linked to feeding [3], narcolepsy and arousal [4, 5], and subsequent studies from our lab showed that the system also plays an important role in reward and reward dysfunctions including addiction [6]. Additional groups showed roles for orexin neurons in cognition, motor activity and stress, as well as cardiovascular, respiratory and other homeostatic functions [7]. The widespread orexin innervation, and multiple functional roles, implies that this system provides a general role in CNS function that contains and integrates many functions. We proposed that the general function for the orexin system is the ability to activate and coordinate adaptive motivational responses to imperative environmental as well as interoceptive signals, a role we termed 'motivational activation'[7]. In this view, the ability of orexin neurons to drive waking and coordinated responses to rewarding stimuli, as well as to regulate homoestatic activities such as waking, reflects engagement of this system to modulate multiple disparate networks in the service of activating adaptive motivational responses to an opportunity and or threat at hand.

Inappropriate activity in such a key behavioral system would, of course, be expected to participate in multiple behavioral disorders. Indeed, evidence indicates that orexins play roles in disorders of stress, affect/emotion and cognitive processes. Here, we focus on a role for this system in responses to highly salient rewards, including drugs of abuse. Early studies found that these neurons not only are activated by, e.g., acute withdrawal from chronic opioids [8, 9], but also that these cells become hyperactivated in response to stimuli that were previously associated with highly salient rewards such as drugs of abuse. Our lab was the first to show that such orexin hyperactivity plays an important role in driving a strong motivational response for previously experienced drugs and is a key element in multiple behaviors that characterize addiction to many drugs including opioids and stimulants, but also salient food [6]. This finding sparked a great deal of additional work that consistently has shown a major role for orexin signaling in reward and reward dysfunctions.

The main topic of this review focuses on a new vista that has appeared in the last few years that substantially extends our understanding of how the orexin system functions in reward and addiction. Recent studies by us and others have found significant plasticity in expression of orexin, such that the numbers of orexin-expressing neurons increase substantially after chronic exposure to highly salient rewards, e.g., drugs of abuse including cocaine [10, 11], opioids [12, 13] and alcohol [14-16]. This increased expression provides a novel source of enhanced signaling in association with such motivational disorders. Below we review evidence for this changed expression, discuss its functional implications, and

posit a novel population of 'reserve' orexin neurons that can be brought to bear for adaptive, but also maladaptive, periods of high motivation.

Motivational Plasticity

Motivation is a critical element in adaptive behavior. Behavioral responding must take into account many factors and become energized (motivated) in one direction competing with others. Although the importance of this construct has been long recognized, defining motivation has been challenging.

Classical fixed-ratio (FR) self-administration or conditioned place preference tasks do not readily translate into measures of motivation. One method of measuring motivation is through increasing FR schedules, where higher ratios require more effort to obtain reward [17]. One widely used version of this is a progressive ratio (PR) schedule, where the FR required to obtain reward increases (usually geometrically) within a session after each reward. The primary result of PR studies is the breakpoint, which is the final ratio performed that resulted in reward within a set criterion time period. Although such schedules capture many elements of motivation for reward, they have serious drawbacks as well: (i) Reward does not occur on each behavioral response and the time between rewards increases during the session and can reach many minutes; this can introduce a delay-discounting confound. (ii) Long periods of time between rewards exerts a demand on working memory in the task, and manipulations that influence working memory could have confounding effects. (iii) Each session typically produces a single result – the breakpoint. Therefore, these tasks have relatively coarse and infrequent data points.

We define motivation operationally using a behavioral economics (BE) approach. This has the advantage of providing a quantitative measure of motivation, separate from other reward-related behaviors such as free consumption or hedonic setpoint (see [18-20] for review of BE methods and capabilities). For many of our studies, we employed a within-session dose-reduction BE procedure in rats [21-23], in which the price (effort) required per mg drug increases in 10min intervals within a session because the dose administered per lever press decreases. We then used an exponential demand equation to fit demand curves (which express consumption as a function of price) to data and allow estimation of the key parameters, α (demand elasticity, inverse of motivation) and Q_0 (amount consumed at nil effort); this procedure is outlined in [18, 24]. For many drugs α and Q_0 are unrelated and vary independently of each other. This is important, as this means the measure of motivation in this procedure (α) is not confounded by changes in consumption (reflected in Q_0). This also is consistent with the fact that α is self-normalizing, as it is the *slope* of the demand curve, which varies independently of the Y-intercept of the curve (which is Q_0). Another advantage of BE over other behavioral procedures for measuring properties such as motivation and hedonic value is that BE does not require working memory and is not confounded by delay-discounting because subjects receive a reward for every lever press. Note that there are other BE

methods that use changes in FR responding to vary effort required per mg drug or fixed unit of other rewards (e.g. food) – these are important for long time-course drugs such as methamphetamine – see [25], or in the case of food, to avoid satiety [26]. Results within drug for dose reduction vs fixed ratio methods are comparable [24].

Plasticity in orexin peptide production

Although a role for the orexin system in motivation has been known for some time, this has conventionally been thought to result from changes in the *activity* of orexin cells. Indeed, orexin neurons exhibit diurnal fluctuations in activity across the day/night cycle, with highest activity during the active period when opportunities to engage in motivated behavior (e.g. feeding, sex, social play) are highest [27, 28]. Moreover, homeostatic challenges that demand adaptive responses, including stress exposure and food deprivation, robustly enhance orexin neuron firing, as does the presentation of stimuli that predict the availability of salient rewards [29-34]. Not surprisingly, therefore, a major goal in addiction neuroscience (and related fields) has been to determine mechanisms driving changes in orexin neuronal excitability [31]. However, an emerging literature now points to an additional, previously underappreciated dimension of orexin system plasticity that contributes to altered motivational states: dynamic and persistent changes in the *number* of orexin-expressing neurons, driven by chronic exposure to highly salient rewarding stimuli.

The number of cells that are immunoreactive for orexin fluctuates in relation to several factors, including time of day (discussed below; [35]). This plasticity in expression likely permits dynamic changes in orexin peptide availability, and in combination with stimulus-driven changes in cell activity, permits adaptive behavioral responses to threats and opportunities (motivational plasticity). Two studies published by independent groups highlighted the possibility that this plasticity in orexin peptide production may be decreased following chronic drug exposure, such that the number of orexin-producing neurons is persistently elevated in addiction. Siegel and colleagues reported a 54% increase in the number of orexin-immunoreactive neurons in postmortem brains from a small number (n=5) of patients with opioid (heroin) use disorder, relative to matched controls [13]. This effect was recapitulated in a dose-dependent manner by daily morphine injections (experimenter-administered) in mice and persisted for up to 4 weeks into withdrawal. Morphine-induced increases in orexin cell numbers was not the result of neurogenesis, as treatment groups did not differ in the number of cells labeled for BrdU or doublecortin, markers of new neurons. In the other study, our group showed a ~25% increase in the number of orexin-immunoreactive neurons in rats after 2 weeks of intermittent cocaine self-administration, a schedule of intake that promotes several addiction-relevant behaviors, including higher drug motivation [10]. Notably, these cell number changes were persistent (differences remained at 5 months after cocaine self-

administration). Moreover, this increased orexin cell number was causally linked to motivational plasticity for drug, as normalization of the number of orexin neurons using a morpholino-antisense approach (~25% knockdown) reduced cocaine demand to levels similar to those observed prior to intermittent cocaine self-administration (although see [36]). (These changes in orexin levels mirror previous demonstrations of drug-induced changes in orexin mRNA levels [37], discussed below). In the time since the publication of these two studies, this phenomenon has been confirmed by several groups, across multiple drugs of abuse, using various models of addiction. One group reported that extended access to cocaine (6h/day, continuous access), which promotes escalation of intake, is associated with a persistent increase in orexin cell numbers in rat [11]. We reported similar increases following intermittent fentanyl self-administration, also in rat [12]. Moreover, in a series of studies in both rats and zebrafish, Leibowitz and colleagues reported increased orexin numbers following prenatal ethanol exposure [14-16, 38]. Notable also is that drugs of abuse do not appear to result in a general upregulation of hypothalamic neuropeptide production, as several of the abovementioned studies reported no change in the number of melanin-concentrating hormone (MCH)-immunoreactive neurons, which are interdigitated, but not overlapping, with orexin neurons. Taken together, several drugs of abuse reliably promote an increase in the number of orexin-immunoreactive neurons, and this is reproducible across several species including human. Moreover, within hypothalamus these effects appear to be specific to orexin-producing neurons, pointing to potentially unique mechanisms underlying their dynamic expression (discussed in more detail below). Notably, similar persistent increases in orexin neuron numbers and mRNA have been observed in animals exposed to high fat diets [39-42], indicating that this phenomenon may reflect a general upregulation of orexin system function in response to highly salient reward.

Topography of orexin plasticity. Interestingly, there is evidence that the upregulation of orexin levels is not uniform across hypothalamus, but rather occurs in a topographically specific manner. Orexin-containing neurons range mediolaterally from the dorsomedial hypothalamus (DMH) through the perifornical area (PF) to the lateral hypothalamus (LH, lateral to the fornix in rat) [2, 3]. In the first paper to directly link the orexin system with reward, our laboratory reported that the activity of orexin neurons in rat LH (‘lateral’ subpopulation), but not DMH/PF (‘medial’ subpopulation), was directly correlated with drug (or food) seeking behavior [6]. In a subsequent study, we reported that medial, but not lateral, orexin neurons were recruited by stress, leading us to hypothesize a functional dichotomy of orexin system function, whereby (at least in rat) medial orexin neurons convey stress/arousal whereas the lateral subpopulation is important for reward [30]. This idea is consistent with the observation that wake-associated Fos activity in orexin neurons is found in the medial but not in the lateral subpopulation [27]. In further support, previous studies had reported that lateral orexin neurons are exclusively activated by antipsychotic-induced weight gain, whereas activity of medial neurons were preferentially associated with

wakefulness and exposure to various stressors [27, 43]. Consistent with this proposed subregional specificity, we showed that the persistent increases in orexin cell numbers following intermittent cocaine self-administration in rat occurred only in LH [10]. This was true also for increased orexin mRNA levels following chronic ethanol consumption in rat (discussed in more detail below; [37]). We also reported that the number of LH, but not DMH/PF, orexin neurons predicts individual variability in cocaine demand elasticity [44]. Moreover, the increased orexin cell numbers in hypothalamus of heroin addicts and morphine-exposed mice were proportionally and statistically greatest in LH [13].

One exception to these observations is a recent study in which we found that increased orexin cell numbers after fentanyl self-administration in rat occurred at a similar magnitude in medial and lateral subregions – a finding we speculate may result from a stronger and more persistent stress phenotype for opioid withdrawal (compared to cocaine) [12]. Intriguingly, data from zebrafish reveal another potential source of topographical specificity; both pre-fertilization and embryonic exposure to ethanol result in increased orexin cell numbers exclusively in the left hemisphere [15, 16]. It is unclear if these effects are species-specific or occur only during early development, as studies examining the effect of drug exposure in rodents and humans, including our own, reported total cell counts across both hemispheres in adults.

Plasticity in orexin function. Intriguingly, we found that increased orexin cell numbers in addiction models were often associated with an increased potency of orexin receptor antagonism. For example, following intermittent access to cocaine or fentanyl, which promoted increased orexin cell expression, suvorexant (dual orexin 1/2 receptor antagonist) or SB-334867 (orexin 1 receptor antagonist) decreased drug demand and cued relapse at doses ~1/3rd of what is needed to have similar effects in short access or FR1 rats [10, 12, 45, 46]. Similarly, a selective orexin 2 receptor antagonist reduced drug intake following daily extended (6h), but not 1h, access to heroin [47], a procedure that promotes an addicted-like state and promotes higher orexin expression [11]. Moreover, when examining individual differences in drug motivation following limited drug access to cocaine or alcohol, we and others routinely have found that animals with high baseline motivation (who likely have higher endogenous orexin cell numbers – discussed below) are most susceptible to the ability of SB-334867 to decrease addiction behaviors [45, 48, 49]. Similar findings have been reported for food, whereby an orexin receptor antagonist reduces motivated responding for highly palatable foods at doses that generally do not affect regular food intake [50-55] (although see [56]). Together, these data indicate that addiction behaviors become more ‘orexin-dependent’ in association with increased orexin expression.

Therapeutic implications. These findings have important potential therapeutic implications - they indicate that orexin receptor antagonists may be able to reduce addiction behaviors at low doses that have limited off-target effects [57, 58]. Indeed, as noted above, the orexin system is critically involved in the regulation of several physiological processes, including feeding and sleep/wake processes, and it would

be clinically important that treatments with orexin antagonists do not interfere with these functions. Studies that have tested this possibility to date are encouraging; in animals that exhibit strong addiction behaviors, SB-334867 inhibits drug seeking at doses that do not interfere with homeostatic feeding [51, 59] or cognition [50, 60]. Moreover, the dual orexin receptor antagonist suvorexant, a compound that is FDA approved for the treatment of insomnia and could readily be repurposed for addiction, can be used with low, non-sedating doses to reduce drug craving in rat [46, 60]. Several clinical trials are currently underway to further characterize the potential utility of such compounds for the management of substance use disorders in the near term [57, 58, 61-65].

Data linking the number of orexin neurons with addiction behaviors following drug exposure ('state' differences) also raises the interesting possibility that individual baseline differences in orexin levels ('trait' differences) may underlie propensity to abuse drugs. Indeed, we recently reported that the number of LH orexin neurons in rat correlates closely with the baseline motivation for cocaine, measured after limited cocaine self-administration training and before the induction of an addiction-like state [44]. We also observed a similar relationship with the ultra-short acting opioid remifentanil (unpublished), again indicating that these findings might be generalized across drugs of abuse. These findings align well with the clinical observation that human narcoleptic patients, which post-mortem studies revealed have 85-95% fewer orexin neurons than normal, rarely develop stimulant abuse, despite long-term treatment with stimulant medications including amphetamines [66, 67] . Unfortunately, there are currently no PET ligand or other techniques for live cranial imaging that allow for estimation of orexin cell number in people; the development of such approaches may have significant value in identifying individuals at risk for developing addictions.

Orexin cell 'reserve': A possible way to modulate motivational gain and direction

A detailed understanding of the mechanisms underlying plasticity in orexin expression remains unknown. We submit here a conceptual framework to foster hypotheses that can be addressed by future studies (**Figure 1**). We propose that the orexin cell field in hypothalamus contains a 'reserve' population of potential orexin neurons that can be transiently recruited under circumstances of high motivation to promote adaptive behavior; by 'recruited', we mean increased orexin peptide production in these 'reserve' cells (discussed below). Recruitment of this reserve population would, for example, promote vigorous food foraging behavior under circumstances of brief caloric deprivation. In the case of drug addiction, however, we postulate that chronic exposure to drugs of abuse *persistently* upregulates orexin peptide production in a select group of neurons, such that the manifestation of 'addiction' is coincident with long-term recruitment of 'reserve' orexin neurons. The result is persistently hypermotivated behavior that is predominantly drug-directed, including – when necessary – the expression of flexible behaviors to

procure drug (see [68]). We predict that this has potential detrimental consequences for other orexin-dependent physiological processes, including reduced homeostatic behaviors such as eating and sleeping.

Plasticity in orexin gene expression is well established. Lawrence and colleagues reported ~15 years ago that chronic ethanol consumption in rat was associated with an increase in LH prepro-orexin mRNA expression [37]. Similarly, repeated nicotine exposure (experimenter administered) increased prepro-orexin mRNA in hypothalamus [69], as did naloxone-precipitated withdrawal in morphine-dependent mice [8]. Importantly, non-drug stimuli have also been shown to promote changes in orexin gene expression. For example, acute food restriction [3], as well as high fat diet exposure [42, 70], promotes increased orexin mRNA levels, as does exposure to stressors [71, 72]. Together, these data indicate that orexin gene expression is normally dynamically altered by exposure to environmental and pharmacologic stimuli – particularly those with motivational relevance.

Despite these well-documented changes in orexin gene expression, little consideration had been given to whether these were linked to changes in orexin peptide production. This question was brought to the fore in 2015 upon the discovery that the number of orexin-immunoreactive neurons in mouse exhibits diurnal fluctuations, such that higher numbers are observed in the active (dark) period compared to the inactive (light) period [35]. These findings indicated that during the light phase, there is a population of neurons that contains the molecular machinery necessary for producing orexin peptide, but are undetectable to an orexin antibody, due to extremely low mRNA or peptide production. Consistent with this interpretation, this same study reported that treatment with colchicine, which promotes the accumulation of neuropeptide in the cell body, resulted in an increase in the overall number of detectable orexin-expressing neurons [35]. Combined with evidence that drug-induced increases in cell numbers may not result from neurogenesis [13], these data point to a ‘reserve’ population of orexin-producing neurons that, under normal circumstances, is recruited to facilitate arousal. It is also notable that the number of detectable MCH neurons did not change following colchicine treatment [35], perhaps pointing to a unique and highly specialized role for orexin neuronal reserves.

We posit that a similar reserve of orexin neurons supports motivational plasticity. Under non-pathological conditions, this reserve is recruited to provide additional orexin peptide in the service of motivational activation, allowing animals to optimally exploit environmental circumstances (**Figure 1b**). During the early phases of addiction, these neurons might be recruited by stimuli that predict drug availability, however this recruitment may be transient and readily overridden by other neural systems (e.g. executive control networks) that control drug use. As drug use becomes more chronic, reserve orexin neurons are repeatedly recruited, but in a way that results in a gradual loss of plasticity in peptide production and a persistent upregulation of their orexin expression (**Figure 1c**). As a result, motivational plasticity is lost, leading to maladaptive and focused hypermotivation for drug.

One additional implication of increased peptide production could be to amplify the effects of firing activity in orexin neurons: more peptide expression may mean more peptide released per action potential. This increased 'gain' in orexin signaling may interact with downstream targets to augment particular elements in behavioral repertoires. We also posit that increased orexin signaling (perhaps via increased receptor number or intracellular signaling mechanisms) accompanies the increased orexin terminals found in specific target areas [11, 47]; this may result in enhanced behavioral impact of orexin input after chronic drug exposure. This, in turn, may account for the abovementioned enhanced potency of orexin receptor antagonists in 'addicted' rats. We note, however, that potential changes in orexin receptor number and signaling needs to be explored further in addiction models.

Dynorphin co-expression. Neurons that produce the orexin peptide also co-express other neurotransmitters including dynorphin [73] which is co-released with orexin at terminal sites [74] and generally has opposing effects to orexin on post-synaptic activity (particularly in VTA) and motivated behavior [75-77]. Cocaine and other drugs increase expression of dynorphin in several reward regions [78]; however, it is unclear whether these changes occur in orexin 'reserve' neurons. It is enticing to hypothesize that exposure to drugs of abuse increase orexin peptide expression while leaving dynorphin levels unaffected, resulting in a net increase in excitatory actions. However, shRNA-mediated alterations in orexin levels are paralleled by compensatory (similar) changes in dynorphin expression in hypothalamus [79], and unpublished studies from our lab indicate that dynorphin increases along with orexin in response to drug exposure. Thus, any imbalance of orexin vs. dynorphin signaling in addiction might be dependent on alterations in their actions at target regions, possibly due to differential release probability, changes in postsynaptic receptor expression, or other signaling properties.

Implications of the orexin 'reserve' hypothesis: future considerations

There are several potential consequences of the proposed orexin reserve described above. The reserve orexin neurons appear to have topographical specificity within hypothalamus (discussed above). We propose that they may also have select targets, so that in response to chronic addictive drugs, orexin expression is increased especially in neurons that project to reward regions where orexin regulates motivated behavior, such as ventral tegmental [42, 80-86] area and nucleus accumbens [87, 88]. This would be consistent with our recent results that orexin innervation of VTA preferentially originates from LH [80]. This arrangement could provide a target-defined specificity/direction to the increased motivation induced by the orexin reserve cells engaged. It is also interesting to consider whether reserve orexin neurons and their related circuits encode motivation that is specific to the drug whose chronic exposure engaged them, or if their enhanced output also alters motivation for other rewards (e.g., food) or drugs of

abuse. Indeed, recruitment of the orexin reserve may serve as a common mechanism underlying high rates of polysubstance abuse [89] and increased risk of overeating disorders [90] in addiction.

Alternatively, it is possible that there exists multiple 'pools' of reserve orexin neurons that drive behavioral responsivity to distinct motivationally relevant stimuli. For example, stress upregulates orexin mRNA, peptide and receptor levels in a way that is functionally linked with stress reactivity [91-94]; this might be achieved via recruitment of unique reserve neurons with preferential input to arousal and emotional centers. The extent to which drugs, stress and other stimuli recruit overlapping vs. distinct reserve orexin neurons should be a focus of studies going forward, especially in view of the large comorbidities among stress, sleep dysregulation and substance use disorders [54, 62, 65].

Mechanisms regulating orexin expression. Other questions relate to the molecular machinery underlying dynamic shifts in orexin gene and protein production, the time scale on which these occur, and how these are perturbed by drugs of abuse. We propose that at baseline, reserve orexin neurons express very little prepro-orexin mRNA, and therefore very little orexin peptide, but can increase both in contexts of high motivational relevance. Some reserve neurons may express little or no mRNA or peptide until stimulated by threat or opportunity. Indeed, it is unclear if there is a stable population of orexin neurons that is supplemented by an additional population of reserve neurons when needed, or if there are many populations that wax and wane in orexin expression depending on the environment and the target of that neuron. Moreover, it is interesting to consider whether reserve neurons co-express dynorphin and other neurotransmitters (e.g. glutamate) when orexin peptide production is dormant.

Several transcription factors have been identified as regulating prepro-orexin gene expression both in vitro and in vivo [95-98], with one (FOXA2) shown to bind the orexin promotor specifically under fasting conditions [96]; it is plausible that a similar mechanism mediates orexin gene (and peptide) upregulation in orexin reserve cells when motivational plasticity is required, and that these processes are dysregulated by chronic drug exposure. Orexin mRNA expression might also be mediated by other mechanisms, including epigenetic modifications (e.g., DNA methylation, histone modifications, chromatin remodeling) and changes in the expression of non-coding RNAs such as microRNAs [99-101]; these possibilities are yet to be fully explored. Recruitment of the 'reserve' orexin neurons may also be activity-dependent, resulting from changes in the balance between excitatory vs. inhibitory inputs arising from specific circuits and cell types [31, 102, 103]. In light of evidence reviewed above indicating that the orexin cell reserve might be more numerous in LH, the mechanisms governing orexin expression may differ for LH versus DMH/PF populations.

Sex differences. Finally, work on sex differences in orexin and addiction is limited. Greater increases in orexin mRNA are observed in female vs. male offspring prenatally exposed to ethanol [38] or chronic stress [91]. Moreover, orexin mRNA concentrations in hypothalamus vary across the estrus cycle,

with higher levels observed in proestrus compared to other phases [104]. These data indicate that a dynamic, drug-sensitive, orexin reserve may exist in females, however more work is required to fully characterize sex differences and their underlying mechanisms.

Conclusions

Additional orexin neurons are observed in response to multiple drugs and across several species, including human, pointing to enhanced orexin expression as a common 'neural signature' of addiction. We propose that this phenomenon reflects a disruption to the normal plasticity of an orexin cell reserve, which, in a non-pathological state is transiently recruited in the service of facilitating adaptive motivational behaviors. In this view, dynamism of this orexin reserve is lost in addiction, such that orexin peptide production is persistently upregulated and behavior becomes hypermotivated, focused and compulsive. The framework outlined here may be a starting point for future efforts to more comprehensively characterize orexin system plasticity in substance use and related disorders.

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Figure Legend

Figure 1. (A) Orexin neuron activity (red line) follows a circadian pattern, with peak activity during the active period. Overlayed on this diurnal activity pattern are phasic bursts during waking; these reflect involvement of the orexin neurons in translating motivationally relevant states into adaptive behavior directed toward managing or exploiting a threat or opportunity, a process we referred to as 'motivational activation'. Figure adapted from [7]. **(B)** Motivational activation is also serviced by state-dependent changes in orexin peptide production. Under non-pathological conditions, a 'reserve' population of orexin neurons with projections to key arousal sites is preferentially recruited during the active period to support wake/arousal. Some reserve neurons with projections to reward centers are also recruited to facilitate adaptive motivated behaviors (e.g., feeding). Recruitment of these reserve cells is primarily governed by circadian factors. Note that non-reserve neurons may also increase their peptide production as a function of behavioral state (not shown here). **(C)** Addiction to drugs is characterized by a persistent and preferential recruitment of reserve orexin reserve neurons that project to reward regions, resulting in sustained, hypermotivated and uncontrolled drug seeking behavior. A loss of plasticity in peptide production by the reserve population also contributes to inappropriate arousal during the inactive period, resulting in sleep dysregulation in addiction.

References

1. Peyron, C., et al., *A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains.* Nat Med, 2000. **6**(9): p. 991-7.
2. de Lecea, L., et al., *The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity.* Proc Natl Acad Sci U S A, 1998. **95**.
3. Sakurai, T., et al., *Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior.* Cell, 1998. **92**.
4. Chemelli, R.M., et al., *Narcolepsy in orexin knockout mice: molecular genetics of sleep regulation.* Cell, 1999. **98**(4): p. 437-51.
5. Lin, L., et al., *The sleep disorder canine narcolepsy is caused by a mutation in the hypocretin (orexin) receptor 2 gene.* Cell, 1999. **98**(3): p. 365-76.
6. Harris, G.C., M. Wimmer, and G. Aston-Jones, *A role for lateral hypothalamic orexin neurons in reward seeking.* Nature, 2005. **437**(7058): p. 556-9.
7. Mahler, S.V., et al., *Motivational activation: a unifying hypothesis of orexin/hypocretin function.* Nat Neurosci, 2014. **17**(10): p. 1298-303.
8. Georgescu, D., et al., *Involvement of the lateral hypothalamic peptide orexin in morphine dependence and withdrawal.* J Neurosci, 2003. **23**(8): p. 3106-11.
9. Sharf, R., M. Sarhan, and R.J. Dileone, *Orexin mediates the expression of precipitated morphine withdrawal and concurrent activation of the nucleus accumbens shell.* Biol Psychiatry, 2008. **64**(3): p. 175-83.
10. James, M.H., et al., *Increased Number and Activity of a Lateral Subpopulation of Hypothalamic Orexin/Hypocretin Neurons Underlies the Expression of an Addicted State in Rats.* Biol Psychiatry, 2019. **85**(11): p. 925-935.
11. Matzeu, A. and R. Martin-Fardon, *Cocaine-Seeking Behavior Induced by Orexin A Administration in the Posterior Paraventricular Nucleus of the Thalamus Is Not Long-Lasting: Neuroadaptation of the Orexin System During Cocaine Abstinence.* Frontiers in behavioral neuroscience, 2021. **15**: p. 620868-620868.
12. Fragale, J.E., M.H. James, and G. Aston-Jones, *Intermittent self-administration of fentanyl induces a multifaceted addiction state associated with persistent changes in the orexin system.* Addict Biol, 2020: p. e12946.
13. Thannickal, T.C., et al., *Opiates increase the number of hypocretin-producing cells in human and mouse brain and reverse cataplexy in a mouse model of narcolepsy.* Sci Transl Med, 2018. **10**(447).
14. Collier, A.D., et al., *Embryonic Ethanol Exposure Affects the Early Development, Migration, and Location of Hypocretin/Orexin Neurons in Zebrafish.* Alcohol Clin Exp Res, 2019. **43**(8): p. 1702-1713.
15. Collier, A.D., et al., *Maternal ethanol consumption before paternal fertilization: Stimulation of hypocretin neurogenesis and ethanol intake in zebrafish offspring.* Prog Neuropsychopharmacol Biol Psychiatry, 2020. **96**: p. 109728.
16. Collier, A.D., et al., *Sexually dimorphic and asymmetric effects of embryonic ethanol exposure on hypocretin/orexin neurons as related to behavioral changes in zebrafish.* Sci Rep, 2021. **11**(1): p. 16078.
17. Roberts, D.C., E.A. Loh, and G. Vickers, *Self-administration of cocaine on a progressive ratio schedule in rats: dose-response relationship and effect of haloperidol pretreatment.* Psychopharmacology (Berl), 1989. **97**(4): p. 535-8.

18. Bentzley, B.S., K.M. Fender, and G. Aston-Jones, *The behavioral economics of drug self-administration: a review and new analytical approach for within-session procedures.* Psychopharmacology (Berl), 2013. **226**(1): p. 113-25.
19. Hursh, S.R. and A. Silberberg, *Economic demand and essential value.* Psychol Rev, 2008. **115**(1): p. 186-98.
20. Oleson, E.B. and D.C. Roberts, *Behavioral economic assessment of price and cocaine consumption following self-administration histories that produce escalation of either final ratios or intake.* Neuropsychopharmacology, 2009. **34**(3): p. 796-804.
21. Zimmer, B.A., E.B. Oleson, and D.C.S. Roberts, *The Motivation to Self-Administer is Increased After a History of Spiking Brain Levels of Cocaine.* Neuropsychopharmacology, 2012. **37**(8): p. 1901-1910.
22. Siciliano, C.A., et al., *Amphetamine Reverses Escalated Cocaine Intake via Restoration of Dopamine Transporter Conformation.* The Journal of neuroscience : the official journal of the Society for Neuroscience, 2018. **38**(2): p. 484-497.
23. Calipari, E.S., et al., *Brief intermittent cocaine self-administration and abstinence sensitizes cocaine effects on the dopamine transporter and increases drug seeking.* Neuropsychopharmacology, 2015. **40**(3): p. 728-35.
24. Bentzley, B.S., T.C. Jhou, and G. Aston-Jones, *Economic demand predicts addiction-like behavior and therapeutic efficacy of oxytocin in the rat.* Proc Natl Acad Sci U S A, 2014. **111**(32): p. 11822-7.
25. Cox, B.M., et al., *Oxytocin Acts in Nucleus Accumbens to Attenuate Methamphetamine Seeking and Demand.* Biol Psychiatry, 2017. **81**(11): p. 949-958.
26. Freeman, L.R., et al., *Sex Differences in Demand for Highly Palatable Foods: Role of the Orexin System.* Int J Neuropsychopharmacol, 2020.
27. Estabrooke, I.V., et al., *Fos expression in orexin neurons varies with behavioral state.* J Neurosci, 2001. **21**(5): p. 1656-62.
28. Mileykovskiy, B.Y., L.I. Kiyashchenko, and J.M. Siegel, *Behavioral correlates of activity in identified hypocretin/orexin neurons.* Neuron, 2005. **46**(5): p. 787-98.
29. Furlong, T.M., et al., *Hypocretin/orexin contributes to the expression of some but not all forms of stress and arousal.* Eur J Neurosci, 2009. **30**(8): p. 1603-14.
30. Harris, G.C. and G. Aston-Jones, *Arousal and reward: a dichotomy in orexin function.* Trends Neurosci, 2006. **29**(10): p. 571-7.
31. Yeoh, J.W., et al., *Activation of lateral hypothalamic group III metabotropic glutamate receptors suppresses cocaine-seeking following abstinence and normalizes drug-associated increases in excitatory drive to orexin/hypocretin cells.* Neuropharmacology, 2019. **154**: p. 22-33.
32. Kurose, T., et al., *Effects of restricted feeding on the activity of hypothalamic Orexin (OX)-A containing neurons and OX2 receptor mRNA level in the paraventricular nucleus of rats.* Regul Pept, 2002. **104**(1-3): p. 145-51.
33. Johnson, P.L., et al., *Orexin 1 receptors are a novel target to modulate panic responses and the panic brain network.* Physiol Behav, 2012. **107**(5): p. 733-42.
34. Moorman, D.E., et al., *Orexin/hypocretin neuron activation is correlated with alcohol seeking and preference in a topographically specific manner.* Eur J Neurosci, 2016. **43**(5): p. 710-20.

35. McGregor, R., et al., *Diurnal fluctuation in the number of hypocretin/orexin and histamine producing: Implication for understanding and treating neuronal loss.* PLoS One, 2017. **12**(6): p. e0178573.
36. Prasad, A.A. and G.P. McNally, *Effects of vivo morpholino knockdown of lateral hypothalamus orexin/hypocretin on renewal of alcohol seeking.* PLoS One, 2014. **9**(10): p. e110385.
37. Lawrence, A.J., et al., *The orexin system regulates alcohol-seeking in rats.* Br J Pharmacol, 2006. **148**(6): p. 752-9.
38. Chang, G.Q., et al., *Moderate Prenatal Ethanol Exposure Stimulates CXCL12/CXCR4 Chemokine System in Radial Glia Progenitor Cells in Hypothalamic Neuroepithelium and Peptide Neurons in Lateral Hypothalamus of the Embryo and Postnatal Offspring.* Alcohol Clin Exp Res, 2020. **44**(4): p. 866-879.
39. Lemus, M.B., et al., *A stereological analysis of NPY, POMC, Orexin, GFAP astrocyte, and Iba1 microglia cell number and volume in diet-induced obese male mice.* Endocrinology, 2015. **156**(5): p. 1701-13.
40. Wortley, K.E., et al., *Orexin gene expression is increased during states of hypertriglyceridemia.* American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 2003. **284**(6): p. R1454-R1465.
41. Morganstern, I., et al., *Increased orexin and melanin-concentrating hormone expression in the perifornical lateral hypothalamus of rats prone to overconsuming a fat-rich diet.* Pharmacol Biochem Behav, 2010. **96**(4): p. 413-22.
42. Borgland, S.L., et al., *Orexin A/hypocretin-1 selectively promotes motivation for positive reinforcers.* J Neurosci, 2009. **29**(36): p. 11215-25.
43. Fadel, J., M. Bubser, and A.Y. Deutch, *Differential activation of orexin neurons by antipsychotic drugs associated with weight gain.* J Neurosci, 2002. **22**(15): p. 6742-6.
44. Pantazis, C.B., et al., *The number of lateral hypothalamus orexin/hypocretin neurons contributes to individual differences in cocaine demand.* Addict Biol, 2020. **25**(4): p. e12795.
45. James, M.H., et al., *Demand elasticity predicts addiction endophenotypes and the therapeutic efficacy of an orexin/hypocretin-1 receptor antagonist in rats.* Eur J Neurosci, 2019. **50**(3): p. 2602-2612.
46. O'Connor, S.L., et al., *The dual orexin/hypocretin receptor antagonist suvorexant reduces addiction-like behaviors for the opioid fentanyl.* bioRxiv, 2020: p. 2020.04.25.061887.
47. Schmeichel, B.E., et al., *Hypocretin receptor 2 antagonism dose-dependently reduces escalated heroin self-administration in rats.* Neuropsychopharmacology, 2015. **40**(5): p. 1123-9.
48. Moorman, D.E., et al., *Orexin/hypocretin-1 receptor antagonism reduces ethanol self-administration and reinstatement selectively in highly-motivated rats.* Brain Res, 2017. **1654**(Pt A): p. 34-42.
49. Mohammadkhani, A., et al., *Orexin-1 receptor signaling in ventral pallidum regulates motivation for the opioid remifentanil.* J Neurosci, 2019.
50. Wiskerke, J., M.H. James, and G. Aston-Jones, *The orexin-1 receptor antagonist SB-334867 reduces motivation, but not inhibitory control, in a rat stop signal task.* Brain Res, 2020. **1731**: p. 146222.

51. Vickers, S.P., et al., *Effects of lisdexamfetamine in a rat model of binge-eating.* J Psychopharmacol, 2015. **29**(12): p. 1290-307.
52. Alcaraz-Iborra, M., et al., *Binge-like consumption of caloric and non-caloric palatable substances in ad libitum-fed C57BL/6J mice: pharmacological and molecular evidence of orexin involvement.* Behav Brain Res, 2014. **272**: p. 93-9.
53. Rorabaugh, J.M., J.M. Stratford, and N.R. Zahniser, *A relationship between reduced nucleus accumbens shell and enhanced lateral hypothalamic orexin neuronal activation in long-term fructose bingeing behavior.* PLoS One, 2014. **9**(4): p. e95019.
54. Mehr, J.B., et al., *Sleep dysregulation in binge eating disorder and "food addiction": the orexin (hypocretin) system as a potential neurobiological link.* Neuropsychopharmacology, 2021.
55. Jupp, B., et al., *The orexin(1) receptor antagonist SB-334867 dissociates the motivational properties of alcohol and sucrose in rats.* Brain Res, 2011. **1391**: p. 54-9.
56. Khoo, S.Y., K.J. Clemens, and G.P. McNally, *Palatable food self-administration and reinstatement are not affected by dual orexin receptor antagonism.* Prog Neuropsychopharmacol Biol Psychiatry, 2018. **87**(Pt A): p. 147-157.
57. James, M.H. and G. Aston-Jones, *Introduction to the Special Issue: "Making orexin-based therapies for addiction a reality: What are the steps from here?".* Brain Res, 2020. **1731**: p. 146665.
58. James, M.H., et al., *Repurposing the dual orexin receptor antagonist suvorexant for the treatment of opioid use disorder: why sleep on this any longer?* Neuropsychopharmacology, 2020.
59. Cole, S., H.S. Mayer, and G.D. Petrovich, *Orexin/Hypocretin-1 Receptor Antagonism Selectively Reduces Cue-Induced Feeding in Sated Rats and Recruits Medial Prefrontal Cortex and Thalamus.* Scientific Reports, 2015. **5**(1): p. 16143.
60. Gentile, T.A., et al., *Effects of Suvorexant, a Dual Orexin/Hypocretin Receptor Antagonist, on Impulsive Behavior Associated with Cocaine.* Neuropsychopharmacology, 2017.
61. Rasmussen, K., D.A. White, and J.B. Acri, *NIDA's medication development priorities in response to the Opioid Crisis: ten most wanted.* Neuropsychopharmacology, 2019. **44**(4): p. 657-659.
62. Campbell, E.J., N.J. Marchant, and A.J. Lawrence, *A sleeping giant: Suvorexant for the treatment of alcohol use disorder?* Brain Res, 2020. **1731**: p. 145902.
63. Campbell, E.J., et al., *Suvorexant to treat alcohol use disorder and comorbid insomnia: Plan for a phase II trial.* Brain Res, 2020. **1728**: p. 146597.
64. Matzeu, A. and R. Martin-Fardon, *Targeting the Orexin System for Prescription Opioid Use Disorder.* Brain Sci, 2020. **10**(4).
65. Fragale, J.E., et al., *The Insomnia-Addiction Positive Feedback Loop: Role of the Orexin System.* Front Neurol Neurosci, 2021. **45**: p. 117-127.
66. Akimoto, H., Y. Honda, and Y. Takahashi, *Pharmacotherapy in narcolepsy.* Dis Nerv Syst, 1960. **21**: p. 704-6.
67. Nishino, S. and E. Mignot, *Pharmacological aspects of human and canine narcolepsy.* Prog Neurobiol, 1997. **52**(1): p. 27-78.
68. Singer, B.F., et al., *Are Cocaine-Seeking "Habits" Necessary for the Development of Addiction-Like Behavior in Rats?* J Neurosci, 2018. **38**(1): p. 60-73.

69. Kane, J.K., et al., *Nicotine up-regulates expression of orexin and its receptors in rat brain.* Endocrinology, 2000. **141**(10): p. 3623-9.
70. Wortley, K.E., et al., *Peptides that regulate food intake: orexin gene expression is increased during states of hypertriglyceridemia.* Am J Physiol Regul Integr Comp Physiol, 2003. **284**(6): p. R1454-65.
71. Chen, X., S. Li, and G.J. Kirouac, *Blocking of corticotrophin releasing factor receptor-1 during footshock attenuates context fear but not the upregulation of prepro-orexin mRNA in rats.* Pharmacol Biochem Behav, 2014. **120**: p. 1-6.
72. Chen, X., et al., *Orexins (hypocretins) contribute to fear and avoidance in rats exposed to a single episode of footshocks.* Brain Struct Funct, 2013.
73. Chou, T.C., et al., *Orexin (hypocretin) neurons contain dynorphin.* J Neurosci, 2001. **21**(19): p. RC168.
74. Li, Y. and A.N. van den Pol, *Differential target-dependent actions of coexpressed inhibitory dynorphin and excitatory hypocretin/orexin neuropeptides.* J Neurosci, 2006. **26**(50): p. 13037-47.
75. Muschamp, J.W., et al., *Hypocretin (orexin) facilitates reward by attenuating the antireward effects of its cotransmitter dynorphin in ventral tegmental area.* Proc Natl Acad Sci U S A, 2014. **111**(16): p. E1648-55.
76. Baimel, C., et al., *Projection-Target-Defined Effects of Orexin and Dynorphin on VTA Dopamine Neurons.* Cell Rep, 2017. **18**(6): p. 1346-1355.
77. Matzeu, A. and R. Martin-Fardon, *Drug Seeking and Relapse: New Evidence of a Role for Orexin and Dynorphin Co-transmission in the Paraventricular Nucleus of the Thalamus.* Frontiers in Neurology, 2018. **9**.
78. Spangler, R., E.M. Unterwald, and M.J. Kreek, *'Binge' cocaine administration induces a sustained increase of prodynorphin mRNA in rat caudate-putamen.* Molecular Brain Research, 1993. **19**(4): p. 323-327.
79. Schmeichel, B.E., et al., *Knockdown of hypocretin attenuates extended access of cocaine self-administration in rats.* Neuropsychopharmacology, 2018.
80. Pantazis, C.B., et al., *Orexin-1 receptor signaling in ventral tegmental area mediates cue-driven demand for cocaine.* Neuropsychopharmacology, 2021.
81. Wang, B., Z.B. You, and R.A. Wise, *Reinstatement of cocaine seeking by hypocretin (orexin) in the ventral tegmental area: independence from the local corticotropin-releasing factor network.* Biol Psychiatry, 2009. **65**(10): p. 857-62.
82. Mahler, S.V., R.J. Smith, and G. Aston-Jones, *Interactions between VTA orexin and glutamate in cue-induced reinstatement of cocaine seeking in rats.* Psychopharmacology (Berl), 2013. **226**(4): p. 687-98.
83. James, M.H., et al., *Orexin-1 receptor signalling within the ventral tegmental area, but not the paraventricular thalamus, is critical to regulating cue-induced reinstatement of cocaine-seeking.* Int J Neuropsychopharmacol, 2011. **14**(5): p. 684-90.
84. Borgland, S.L., E. Storm, and A. Bonci, *Orexin B/hypocretin 2 increases glutamatergic transmission to ventral tegmental area neurons.* Eur J Neurosci, 2008. **28**(8): p. 1545-56.
85. Borgland, S.L., et al., *Orexin A in the VTA Is Critical for the Induction of Synaptic Plasticity and Behavioral Sensitization to Cocaine.* Neuron, 2006. **49**(4): p. 589-601.
86. Mehr, J.B., M.M. Bilotti, and M.H. James, *Orexin (hypocretin) and addiction.* Trends Neurosci, 2021.

87. Lei, K., et al., *Nucleus Accumbens Shell Orexin-1 Receptors Are Critical Mediators of Binge Intake in Excessive-Drinking Individuals.* Front Neurosci, 2019. **13**: p. 88.
88. Lei, K., et al., *Nucleus Accumbens Shell and mPFC but Not Insula Orexin-1 Receptors Promote Excessive Alcohol Drinking.* Frontiers in Neuroscience, 2016. **10**(400).
89. James, M.H., et al., *The orexin (hypocretin) neuropeptide system is a target for novel therapeutics to treat cocaine use disorder with alcohol coabuse.* Neuropharmacology, 2020. **183**: p. 108359.
90. Escrivá-Martínez, T., et al., *Binge Eating and Binge Drinking: A Two-Way Road? An Integrative Review.* Curr Pharm Des, 2020. **26**(20): p. 2402-2415.
91. Grafe, L.A., et al., *Orexins Mediate Sex Differences in the Stress Response and in Cognitive Flexibility.* Biol Psychiatry, 2017. **81**(8): p. 683-692.
92. Kim, T.K., et al., *Antidepressant effects of exercise are produced via suppression of hypocretin/orexin and melanin-concentrating hormone in the basolateral amygdala.* Neurobiol Dis, 2015. **79**: p. 59-69.
93. Yaeger, J.D.W., et al., *Orexin 1 Receptor Antagonism in the Basolateral Amygdala Shifts the Balance From Pro- to Antistress Signaling and Behavior.* Biol Psychiatry, 2022.
94. Gyawali, U. and M.H. James, *Orexin (Hypocretin) Signaling in the Basolateral Amygdala Contributes to Individual Differences in Stress Sensitivity.* Biological Psychiatry, 2022. **91**(9): p. 775-777.
95. Tanaka, S., *Chapter Five - Transcriptional Regulation of the Hypocretin/Orexin Gene*, in *Vitamins & Hormones*, G. Litwack, Editor. 2012, Academic Press. p. 75-90.
96. Silva, J.P., et al., *Regulation of adaptive behaviour during fasting by hypothalamic Foxa2.* Nature, 2009. **462**(7273): p. 646-650.
97. Tanaka, S., et al., *Transcriptional regulation of the hypocretin/orexin gene by NR6A1.* Biochemical and Biophysical Research Communications, 2010. **403**(2): p. 178-183.
98. Honda, M., et al., *IGFBP3 colocalizes with and regulates hypocretin (orexin).* PLoS One, 2009. **4**(1): p. e4254.
99. Nestler, E.J. and C. Lüscher, *The Molecular Basis of Drug Addiction: Linking Epigenetic to Synaptic and Circuit Mechanisms.* Neuron, 2019. **102**(1): p. 48-59.
100. Bali, P. and P.J. Kenny, *Transcriptional mechanisms of drug addiction* . Dialogues Clin Neurosci, 2019. **21**(4): p. 379-387.
101. Kenny, P.J., *Epigenetics, microRNA, and addiction.* Dialogues Clin Neurosci, 2014. **16**(3): p. 335-44.
102. Rao, Y., et al., *Repeated in vivo exposure of cocaine induces long-lasting synaptic plasticity in hypocretin/orexin-producing neurons in the lateral hypothalamus in mice.* J Physiol, 2013. **591**(Pt 7): p. 1951-66.
103. Yeoh, J.W., et al., *Cocaine potentiates excitatory drive in the perifornical/lateral hypothalamus.* J Physiol, 2012. **590**(Pt 16): p. 3677-89.
104. Porkka-Heiskanen, T., et al., *Orexin A and B levels in the hypothalamus of female rats: the effects of the estrous cycle and age.* Eur J Endocrinol, 2004. **150**(5): p. 737-42.