

Recent perspectives on orexin/hypocretin promotion of addiction-related behaviors

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Abstract

The neuropeptide hypocretin/orexin plays a broad and important role in physiological functions ranging from addiction, stress, and anxiety to sleep, energy metabolism, and homeostatic regulation. A number of recent reviews addressing the importance of orexin for different addictive behaviors, especially the contribution of orexin-1-receptors (Ox1Rs) in responding for intoxicants in higher-motivation individuals and situations, and orexin-2-receptor (Ox2Rs) in stress-related aspects of addictive responding. This may parallel the importance of more lateral orexin neurons in the hypothalamus for reward and more medial for stress and arousal. However, there is clearly also some crossover, which may reflect, in part, where positive and negative conditioning (reward- and relief-seeking) are both present concurrently in established addiction, and also where orexin signaling can differ in subregions of a particular brain region. Here, we attempt to examine and

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synthesize some of the most recent work addressing orexin functions in addiction, including a particular role for Ox1Rs for driving responding in higher-motivation individuals and under higher levels of effort. While there are some commonalities across addictive substances addressed here (alcohol, cocaine, opiates), there are also some differences, which may relate to several factors including the speed of intoxication with a given substance. Together, recent findings have shed important insight and clues into what a more unified role of Ox1Rs might entail, and critical areas for future work. In addition, these many studies support the development of Ox1R blockers for use in humans to counteract addiction and other disorders of motivation.

Introduction

Purpose of this Review

Here, we examine and attempt to synthesize some of the most recent work addressing the function of the neuropeptide orexin, especially studies related to addiction, and to identify critical areas where additional information is needed. One particular focus is on the particular importance of orexin for responding for more motivating substances, especially when overcoming cost and higher effort are required. Similarly, orexin receptors are particularly impactful in individuals with higher motivation for and intake of intoxicants. Orexins are important for anxiety and stress responding, perhaps suggesting a role in certain higher salience behaviors, rather than positive or negative events per se. Also interesting are differences in orexin regulation of behavior in females and males, e.g. where females have higher orexin levels and differential regulation of orexin-related behaviors. We also try to address the molecular bases of differential orexin effects,

including changes in the number of orexin-expressing neurons. Finally, we briefly address the targeting of orexin receptors as possible therapy to reduce the impact of addiction.

Basics of Orexin Signaling

The neuropeptide hypocretin/orexin has been written about extensively, with a number of recent reviews addressing the importance of orexin for different addictive behaviors as well as negative affective states (e.g. Johnson et al., 2012; Barson and Liebowitz, 2016; James et al., 2017a,b; Walker and Lawrence, 2017; Anderson et al., 2018; Moorman, 2018; Summers et al., 2018; James and Aston-Jones, 2020). More generally, orexins play an important role in physiological functions ranging from addiction, stress, and anxiety to sleep, energy metabolism, and homeostatic regulation (reviewed in de Lecea et al., 1998; Mahler et al., 2014; Brown et al., 2015b; Li et al., 2016; James et al., 2017a,b). Orexins are synthesized in a specific subset of cells localized to the lateral hypothalamus area (LHA) and regions just medial (dorsomedial hypothalamus, perifornical area), while orexin-containing fibers are broadly distributed across the brain allowing very wide potential impact. Also, LHA are proposed to mediate reward behaviors, while more medial orexin neurons may be more important for stress and arousal (Harris et al., 2005; Aston-Jones et al., 2010; Freeman et al., 2018). Further, orexins act through two classes of receptors, orexin-1-receptor (Ox1R) and orexin-2-receptor (Ox2R). Ox2Rs are more broadly distributed across the brain, while Ox1Rs are more specifically localized within key limbic regions (e.g. D'Almeida et al., 2005), and, in parallel, Ox2Rs are related more to sleep and arousal while Ox1Rs are more related to addiction, reward and motivation, although there clearly is some crossover (de Lecea et al., 1998; Mahler et al., 2014; Brown et al., 2015b; Li et al., 2016; James et al., 2017a,b) (and see below).

Orexin importance for more motivating substances and/or required effort

One central finding is that orexin signaling is of particular importance for driving many motivation- and addiction-related behaviors (Aston-Jones et al., 2010; Mahler et al., 2012; Boutrel et al., 2013; Mahler et al., 2014; Barson and Leibowitz, 2016; James et al., 2017b). More specifically, Ox1Rs are linked to ostensibly higher-motivation behaviors, with less impact on intake involving less reinforcing substances and/or simpler response requirements. For example, using SB336847 (SB), a generally validated Ox1R-targeted compound (see Summers et al., 2018), Ox1Rs are shown to be critical for cost-resistant but not cost-free responding for cocaine and some opiates (described in detail below). In addition, Ox1R inhibition reduces progressive-ratio responding for cocaine and high-fat food but not simpler food pellets (Borgland et al., 2009), although response levels are similar for food and cocaine. Also, work from our lab and others finds greater Ox1R importance in individuals that drink higher levels of alcohol, with parallel studies for cocaine and opiates (see below). While many critical questions remain, a central Ox1R role in higher motivation suggests that orexin receptor blockers may reflect useful therapeutic interventions for addiction and other motivational disorders in humans (Khoo and Brown, 2014; Li et al., 2016; James et al., 2017b; Walker and Lawrence, 2017; Perry and Zhang, 2018).

One area of mixed findings is the impact of Ox1R inhibition on responding for natural rewards. In some studies, responding for high-fat and/or -sweet substances requires Ox1Rs (e.g., Borgland et al., 2009; Srinivasan et al., 2012; Anderson et al., 2014; Cason and Aston-Jones, 2014; Olney et al., 2015), while in others it does not (e.g., Lopez et al., 2016; Matzeu and Martin-Fardon, 2018; Fragale et al., 2019). Indeed, one group found that cocaine but not highly palatable foods increases cFos in orexin neurons (Matzeu and Martin-Fardon, 2018). Also, Jupp and colleagues

(2011) show that SB reduces alcohol and sucrose responding under a simpler response requirement (fixed-ratio 3) but only impact alcohol (not sucrose) responding under progressive ratio responding (and somewhat converse to the general link of Ox1R to higher motivation). Further, many findings indicate that Ox1Rs in particular brain regions are required for drug/alcohol but not sucrose intake. Local Ox1R inhibition with SB in cortex reduces nicotine but not food intake (Hollander et al., 2008) and alcohol but not sucrose seeking (Brown et al., 2015a), and SB in VTA reduces alcohol seeking, cocaine intake, and morphine CPP but not locomotion (Aston-Jones et al., 2010; James et al., 2011; Mahler et al., 2013; Brown et al., 2015a). Furthermore, i.c.v. administration of SB decreases alcohol but not saccharin or food intake (Carvajal et al., 2015). Also, higher-drinking selected mice have higher anxiety and compulsion-like alcohol responding, with no difference in saccharin preference or sweet or bitter taste reactivity (Wolstenholme et al., 2011; Radwanska and Kaczmarek, 2012; Bahi, 2013). Thus, one critical question is how higher motivation for addictive substances might differ from higher motivation for “natural” rewards. One speculation is that natural reward circuitry taps into more basic homeostatic regulatory systems, while addiction involves dysregulation of emotional processing rather than physiological homeostasis per se. In addition, sensory, attentional, response, and reward aspects of the context present could all influence whether orexins are required to respond. For example, the presence or absence of food deprivation might also be important, since it increases orexin cell activity (e.g. Soya et al., 2017; Yeoh et al., 2019; but see Cason and Aston-Jones, 2013). Thus, orexin importance for natural versus drug rewards remains an area needing strong clarification.

Some Technical Considerations when Studying Orexin

One avenue of particular interest is the identification of behaviors that are sensitive or insensitive to orexin receptor inhibition, which could provide important clues to understanding more specifically the central role of orexin, including Ox1Rs. There are a few caveats to this approach, including that the majority of Ox1R studies utilize SB, which, although widely used, has the possibility of off-target effects especially at higher doses. However, some groups have argued that the behavioral specificity of SB indicates a selective Ox1R effect, e.g. if there are limited effects on simpler motor behaviors but more selective effects on particular aspects of motivated responding. As discussed below, this approach has resulted in some mixed findings, but are nonetheless overall quite encouraging. Also, some central findings have been replicated with additional Ox1R specific drugs for alcohol intake (e.g. Lopez et al., 2016), or with Ox1R shRNA for cocaine intake (Bernstein et al., 2018), increasing confidence in the selectivity of SB in targeting Ox1R. In addition, since SB can impact Ox2Rs at higher concentrations (Scammell and Winrow, 2011), studies can show a behavioral effect of SB but not an Ox2R blocker (e.g., Lei et al., 2016a; 2016b), also increasing confidence in the selectivity of any observed SB effect (at least in relation to different orexin receptors).

Also of interest is the relative dose effect of Ox1R inhibition, i.e., where some behaviors are only inhibited by higher levels of SB, while others can be inhibited by much lower SB concentrations. For example, compulsion-like responding for alcohol is inhibited by lower SB doses (systemically) relative to those that reduce alcohol-only intake (Lei et al., 2016a, see below). We interpret such a finding to indicate that the behavior is more dependent on the particular signaling pathway (in this case orexin), with fewer parallel signaling systems to help support the behavior. However, one caveat when evaluating SB studies is that the solubility of commercially available SB may have changed across time. As strange as it may seem, studies after the 2000s

require DMSO in order to get SB to be soluble, while studies before this time report solubility in vehicle without DMSO (discussed in Lei et al., 2019). While the exact basis of this remains unclear, it represents an important consideration when comparing the results of earlier versus later studies using SB. However, despite these different concerns, studies using systemic SB and other Ox1R blockers have shown interesting and selective patterns of behavioral disruption (described in detail below).

Another, more theoretical, caveat when searching for a unifying role for orexin is that many studies involve systemic drug administration. In particular, given the wide range of states orexin is implicated in, systemic inhibition might decrease both activating and inactivating pathways, and thus have no clear net effect. While this seems more complicated, some experimental findings (not related to orexins) suggest such a possibility. For example, Yun and colleagues (2004) find that dopamine receptor blockers in the nucleus accumbens (NAc) strongly reduce responding to a predictive cue, while glutamate receptor blockers or tetrodotoxin in the NAc do not reduce cue responding. One explanation for such a finding is that the NAc contains direct and indirect output pathways that act in opposition (e.g. as suggested by Calipari et al., 2016, and others). Thus, strong inhibition would remove the impact of both output pathways, resulting in no net effect, while a more selective disruption of signaling (dopamine receptors) would impact only one of the two outputs and thus is able to have a behavioral effect. Similarly, we find that inhibiting NMDA receptors within the NAc disrupts compulsion-like alcohol drinking, with no effect on alcohol-only intake (Seif et al., 2013; 2015), but that strong NAc suppression with muscimol/baclofen did not reduce either form of drinking (unpublished findings). Given the diversity of sites in the brain where orexin acts, and the ability to regulate both appetitive behaviors and anxiety/stress related

avoidant behaviors (see Introduction), some caution may be warranted for studies with systemic receptor inhibitors.

Addiction and responding in the face of negative consequences

Compulsion-like drives, where consumption continues despite negative consequences, is central to allowing much of excessive human intake (Larimer et al., 1999; Anton, 2000; Tiffany and Conklin, 2000; Koob and Volkow, 2010; Hopf and Lesscher, 2014; Everitt and Robbins, 2016; Voon et al., 2016). The recent DSM-5 emphasizes disordered choosing (deciding to continue intake despite known harm) as central to the diagnosis of alcohol use disorder (DSM-5, 2013; Grant et al., 2015). This cost/effort-resistant aspect of intake is likely critical for driving addiction for many substances, in concert with other factors such as greater valuation of rewards (Hogarth, 2020). There have been different considerations of compulsion, some of which consider compulsion a stronger form of devaluation-resistant habit (Everitt and Robbins, 2016), while others consider compulsion more of a goal-directed process where habit mechanisms may be recruited in the service of overcoming and ignoring conflict associated with action (see Tiffany and Conklin, 2000; Naqvi and Bechara, 2010; Hopf and Lesscher, 2014, Hopf, 2017). There is still some divergence in consideration of whether consequence-resistant actions involve excessive insensitivity to cost information (e.g., Claus et al., 2013; Reiter et al., 2016; Sebold et al., 2017) or not (Hogarth and Hardy, 2018; Hogarth, 2020). However, in either case, some aspect of cost discounting seems to be a consistent consideration for many addicts (references above). Thus, even with these caveats, there is considerable interest in understanding the underlying circuitry and molecular signaling that allows aversion-resistant drives to have such power in promoting intake. Also, since compulsion-like determination likely involves high motivation (since the drive to

consume persists despite negative consequences), it is also logical that Ox1Rs might be particularly important for promoting aversion-resistant responding (as discussed below). Furthermore, tolerance of greater relative effort (as described below) is likely to be conceptually related to cost-resistant responding, and thus a more specific role for Ox1Rs might also be predicted for higher effort conditions.

Orexins and alcohol: Ox1Rs preferentially drive intake in higher-drinking individuals

A number of findings converge on the idea that Ox1Rs are critical for driving higher-motivation responding for alcohol. Systemic inhibition of Ox1Rs specifically reduces alcohol intake in higher-drinking individuals, with little impact in individuals with lower basal intake levels; this is seen using alcohol intake from a bottle (Moorman and Aston-Jones, 2009; Alcaraz-Iborra et al., 2017) and using operant-based methods where animals lever press to receive alcohol (Moorman et al., 2017). This suggests that Ox1Rs may be particularly important for driving excessive motivation in higher-intake drinkers, and thus might be a potent therapeutic intervention to reduce the harm of addictive drives. Indeed, binge alcohol consumption is particularly harmful (e.g., CDC, 2014; GBD 2016 Alcohol and Drug Use Collaborators, 2018), making excessive intake a particularly important clinical target for therapeutic interventions, and of interest to our lab and others examining the importance of orexin signaling.

To better understand how Ox1Rs can promote high consumption, it is critical to identify the brain regions where Ox1Rs act to drive excessive intake. Indeed, we find that inhibiting Ox1Rs in NAc Shell or the vmPFC is effective in reducing alcohol bingeing in adult male C57BL6/J mice (Lei et al., 2016b; Lei et al., 2019), and other work implicates CeA and VTA Ox1Rs for mouse

alcohol intake (Olney et al., 2017); in contrast, Ox2R inhibition in NAc Shell does not alter alcohol drinking, and Ox1R inhibition in NAc Shell does not reduce saccharin intake (Lei et al., 2016b). In addition, we observe that inhibiting NAc Shell Ox1Rs or more global activity (using GABA receptor agonists) significantly decreases mouse alcohol intake under two different bottle-based alcohol drinking paradigms, a Limited Daily Access model (2 hours per day) and an Intermittent Access Model (three overnight alcohol access sessions per week, with at least 24 hours per drinking period) (Lei et al., 2016b; 2019). Thus, Shell activity, orexin-mediate and more global, appears relevant across alcohol drinking models, at least in mice. Importantly, given the large data set we had, we combined results from different drinking conditions and inhibition methods, and demonstrated that inhibiting Shell Ox1Rs or global activity is particularly effective at reducing the higher intake levels in excessive-drinking mice, with no average effect in more moderate bingers (Lei et al., 2019). Thus, we identify the Shell as an important site where Ox1Rs drive the excessive alcohol intake in higher-drinking individuals. We also demonstrate that intake in moderate bingeing individuals can be reduced, using systemic administration of the GABAB agonist baclofen. This indicates that a lack of regulation by Shell Ox1Rs and global activity in moderate bingers is not due to a floor effect. In addition, we find a lack of effect of NAc Shell SB on saccharin intake (Lei et al., 2016b), in agreement with studies showing that altering Shell activity suppresses intake of alcohol but not sweet fluid (Stratford and Wirtshafter, 2011; Rewal et al., 2012; Kasten and Boehm, 2014; Lum et al., 2014), and where Shell Ox1R inhibition does not impair chow intake or locomotor activity (Thorpe and Kotz, 2005; Kotani et al., 2008; Qi et al., 2013). Thus, our results strongly suggest that the Shell is a critical region where Ox1Rs promote excessive alcohol intake in higher drinking-individuals, providing the first link between a specific brain signal (NAc shell Ox1Rs) and orexin regulation of intake in excessive drinkers. In addition, although there are fewer

studies, Ox1Rs in ventral mPFC are important for binge alcohol drinking (Lei et al., 2016b) and also critical for cue potentiation of feeding (Cole et al., 2020).

We note that there have been mixed results regarding the presence of Ox1Rs in the NAc, e.g. with some studies finding little evidence for NAc Ox1Rs by immunostaining (Marcus et al., 2001). However, our (Lei et al. 2016b) and other studies (Thorpe and Kotz, 2005; Kotani et al., 2008; Qi et al., 2013) provide functional evidence for Ox1Rs in NAc, even if they are low abundance relative to other areas. In addition, *in situ* studies (e.g. D’Almeida et al., 2015) and our unpublished RT-PCR work find low but measurable levels of Ox1R mRNA within the medial NAc.

Overcoming cost to get alcohol: cost-resistant responding is sensitive to lower doses of Ox1R blockers

As noted above, compulsion-like drives, where consumption continues in the face of negative consequences, is an important contributor to human addiction. Interestingly, we discovered that compulsion-like alcohol intake in mice, where mice continue to drink alcohol containing the bad-tasting quinine, is inhibited by lower doses of Ox1R inhibitor (SB 3 mg/kg, given systemically) (Lei et al., 2016a), where much higher concentrations of inhibitor are needed to reduce alcohol-only, ostensibly non-compulsive alcohol drinking (SB 30 mg/kg, Anderson et al., 2014). Thus, Ox1R blockers might also represent a particularly useful therapeutic intervention for compulsion-like as well as excessive motivation to drink alcohol (see Khoo and Brown, 2014; Li et al., 2016; James et al., 2017b; Walker and Lawrence, 2017; Perry and Zhang, 2018). While there are some caveats when comparing the dose response profile of Ox1R inhibition (see above),

nicotine self-administration (Hollander et al., 2008) and alcohol intake in genetically alcohol-preferring rats (Jupp et al., 2011; Anderson et al., 2014) are also inhibited by low SB doses given systemically. Both behaviors might be considered to reflect higher motivation states, supporting the overall speculation that Ox1Rs become more and more critical as the subjective salience of the reinforcer increases. We also note that there are some differences among extant mouse studies: Anderson et al. (2014) find that SB at 30 but not 10 mg/kg reduces alcohol-only intake, and we find that SB at 10 and 3 mg/kg do not reduce alcohol-only drinking but do decrease compulsion-like intake (Lei et al., 2016b), while Olney et al. (2015) find that SB at 5 and 10 mg/kg do reduce mouse alcohol-only consumption. The reasons for such discrepancies remain unclear, since there may be technical differences across studies. In particular, this highlights the importance of comparing aversion-resistant and alcohol-only intake in the same study, e.g. in ours showing inhibition of compulsion-like drinking with lower SB doses (Lei et al., 2016a).

Orexins and cocaine: Ox1Rs in higher-motivation individuals and for higher cost

A substantial number of studies, many from Gary Aston-Jones and colleagues, have examined how Ox1Rs mediate different cocaine-related behaviors. Together, they strongly support the central orexin role in higher motivation individuals and situations, with some important nuances. Before considering these findings, we will describe a powerful method called the behavioral economic approach. Using this, one can determine motivation for cocaine in the absence of response cost and with increased levels of cost in the same session (e.g., James et al., 2019a). In practice, this involves requiring an animal to lever press to get infusion of cocaine, and, importantly, the dose of drug for a given amount of responding is decreased across the session.

This allows two values to be calculated. One is Q_0 , the amount of responding when there is no cost (i.e., where responding gives a relatively high dose of drug, which occurs at the onset of the session). The other is alpha, an index of “demand elasticity,” which indicates how much responding is reduced as the level of drug per response goes down, and thus the relative price of responding increases. An increase in alpha indicates a decrease in motivation: higher demand elasticity (alpha) means that drive to respond is more elastic, i.e. influenced and reduced by increasing cost. Another way to consider this is where lower alpha indicates lower elasticity, meaning that demand and responding are not as influenced when the effort-to-reward ratio increases. Thus, one can determine the basal desire for a drug as well as willingness to overcome cost to get a drug in the same session. The method is particularly useful for drugs where a smaller unit dose can produce a measurable impact (e.g., cocaine and opiates).

To better understand the relation between orexin signaling and cocaine motivation, recent work has compared cocaine responding under different access models, including Long daily Access (LgA, 6 hours/day), Short daily Access (ShA, 1 hour/day), and Intermittent Access (InA, brief periods of access within LgA) (see James et al., 2019a; 2019b). These models all involve lever-pressing to get cocaine infusions, but longer access to self-administer within a day (LgA, InA) leads to escalation of intake across days and other changes indicating development of an addiction-like state, which is observed for a number of addictive substances (see Ahmed and Koob, 1998). While LgA increases intake relative to other groups, InA leads to development of a cluster of addiction-related behaviors, including greater cost-resistance (lower demand elasticity), cued reinstatement (renewed responding when the drug-paired cue is presented), and appearance of negative mood (James et al., 2019a). In addition, lower doses of Ox1R blocker (SB 10 mg/kg) reduce drug-related behaviors after InA, while higher doses (SB 30 mg/kg) are needed in other

exposure groups. Interestingly, and discussed more below, InA also leads to long-term increases in the number of LHA orexin neurons, likely contributing to increased cocaine motivation (James et al., 2019b). In agreement, decreasing orexin expression in the LHA decreases cost-resistant motivation for cocaine without impacting responding under minimal cost (Pantazis et al., 2019); interestingly, less efficient orexin knockdown correlates with higher retention of motivation for cocaine. We note that another study finds that reducing orexin levels reduces LgA but not ShA cocaine self-administration (Schmeichel et al., 2018), with no effect on a number of other measures including food intake, body weight, locomotion, elevated plus maze (an anxiety measure), or stress response to forced swim (a depression-like measure). Together, these findings indicate that self-administration regimes that elevate motivation for and intake of cocaine can do so in an Ox1R-dependent manner. In agreement, Ox1R inhibition decreases cocaine cued reinstatement, with a larger effect under conditions associated with higher motivation (James et al., 2019a; 2019b). These behavioral economics studies also somewhat mirror previous work showing Ox1R importance for progressive ratio responding but not under simpler response requirements (Borgland et al., 2009; Mahler et al., 2012; 2014), although Ox1R can inhibit cocaine responding with simpler responding in some studies (Bernstein et al., 2018).

In addition to paradigm-related changes in cocaine motivation, there are also important individual differences. Animals whose basal cocaine self-administration is less sensitive to cost also exhibit greater motivation under other behavioral measures, and greater involvement of Ox1R signaling. For example, animals that exhibit cost-resistance (lower alpha) early in their self-administration history also exhibit several addiction-like behaviors after a much longer self-administration history, including greater punishment resistance (compulsivity), responding in the absence of reward (i.e. responding under extinction), and cued reinstatement. Importantly,

responding in these cost-resistant individuals is also more sensitive to Ox1R antagonists (Bentzley and Aston-Jones, 2015; James et al., 2019a; 2019b). Thus, cost-resistance early in cocaine intake (perhaps an innate difference in motivation) likely reflects an Ox1R-related addiction endophenotype. We note that this early cost-resistance biomarker predicts lower future demand elasticity after either LgA or InA intake, indicating that innate cocaine motivation and intake-related enhancement of cocaine drive (described above) might be parallel but somewhat separable contributions. Nonetheless, first day drinking of alcohol predicts consumption level weeks later (Lei et al., 2019), with Ox1R inhibition predominantly reducing consumption in higher-consuming individuals (see above). Together, these support the idea that individuals may possess innate or quickly developed, Ox1R-mediated drive for intoxicants, which can be detected early in an addiction career as a biomarker for future addiction risk.

One question about the behavioral economic approach is whether the cost (getting less drug for the same response) engenders aversion. We think this is critical, since there could be different circuit mechanisms when overcoming aversion relative to overcoming a cost that doesn't evoke aversion. In the case of cocaine, several groups have shown that a cue paired with delay in cocaine delivery can be aversive, e.g. eliciting a place aversion (Wheeler et al., 2015). Since the animal undergoing behavioral economics likely can detect interoceptively that it is getting a smaller amount of cocaine, this could be akin to delaying the cocaine effect and thus elicit aversion. We believe such considerations are important when trying to disambiguate different pathological mental states associated with expression of addiction, and the particular role of Ox1Rs. In addition, as discussed in greater detail below, it is likely that addiction be driven by both positive (drug reward) and negative (seeking of relief from negative affect) factors, which could be present

concurrently. While this might be complicated to disentangle, it is crucial when trying to understand mechanisms at play in human addicts and their ostensibly more complex inner worlds.

Orexins and overcoming cost to get opiates

A number of interesting studies have also found that Ox1Rs regulate opiate-related motivation. Interestingly, some opiates show a similar pattern as cocaine, while others show a different relation between orexin and motivation. This is consistent with other findings of differing mechanisms for cocaine and opiates (cf Badiani et al., 2011; Porter-Stransky et al., 2017).

Fentanyl is a potent and widely abused opiate, and inhibiting Ox1Rs with SB (30 mg/kg) increases alpha (decreases cost-resistance) without changing Qo (basal intake), suggesting that Ox1Rs maintain lever pressing for fentanyl intake as cost increases but do not regulate responding under minimal cost (Fragale et al., 2019; SB 10 and 3 mg/kg have no effect). Interestingly, the inhibitory effect of 30 mg/kg SB was more pronounced in individuals with higher cost-resistance (lower alpha), and these same individuals also had greater cued reinstatement, which was blocked by SB 30 (but not SB 10) mg/kg). As controls, 30 mg/kg SB did not change lever pressing for sugar pellets or cued sucrose reinstatement. These findings concur with the pattern for cocaine, where Ox1Rs are more important for driving responding in higher-motivation individuals. Also, fentanyl Qo and alpha were not related across individuals, providing evidence that low-challenge intake and cost-resistant intake may be driven by dissociable mechanisms, which has also been reported for cocaine (Belin and Deroche-Gamonet, 2012).

Studies of a different opiate, the short-acting remifentanyl, find a different contribution for Ox1Rs (Porter-Stransky et al., 2017; Mohammadkhani et al., 2019a; 2019b). For this

compound, intake in higher takers, who are also more shock resistant, is not regulated by systemic inhibition of Ox1Rs. In contrast, in individuals who are lower takers, Ox1R blockers reduce cost-free intake (Qo) and cued reinstatement (Porter-Stransky et al., 2017). Thus, Ox1Rs seem to play a preferential role in promoting intake in lower-intake individuals for remifentanyl. In addition, inhibiting Ox1Rs specifically in the ventral pallidum decreases low-cost consumption of remifentanyl, and SB is more effective as motivation increases (Mohammadkhani et al., 2019a). Interestingly, Ox1R inhibition in VP or systemically leads to longer-term inhibition of remifentanyl responding (Mohammadkhani et al., 2019a; 2019b), with reduced behavioral responding up to 48 hours after drug exposure; while the exact reason for this is unclear, it has the implication that orexin receptors would require less frequent dosing, with reduced potential for side effects, and also of value since repeated SB can alter subsequent response to SB (Zhou et al., 2012). Finally, the reason for a varied Ox1R role for different opiates is unclear, but one speculative possibility is that the balance between positive and negative effects may differ, e.g. leading to differential Ox1R contributions due to different anxiety versus hedonic impacts (see following section). In particular, shorter versus longer acting opiates could have different experiential and mnemonic effects for both the positive intoxication and negative effects during withdrawal. These possibilities remain speculative for now, but are an interesting avenue for future work.

Molecular Differences among High and Low Intake Individuals

Future experiments are needed to address whether there are different molecular factors in the Shell, such as Ox1R levels, that promote intake specifically in higher-drinking individuals. One challenge to such studies is that biochemical measures are performed after weeks of drinking

(e.g. as in Alcaraz-Iborra et al., 2017), and higher drinkers have experienced more alcohol. This could reflect an insurmountable confound, with the possibility of different intoxicant-related adaptations in addition to any innate differences in drive for alcohol. Nonetheless, we find that intake levels in the first drinking session predict consumption levels weeks later, opening the possibility that individual differences in drive to consume could be determined by a single drinking session (Lei et al., 2019). However, the level of alcohol exposure would still differ across individuals, and a single drinking session can produce substantial molecular changes (Beckley et al., 2016). Another solution may involve the behavioral economics methods described above, where animals respond for smaller and smaller levels of the intoxicant across the session, and where willingness to respond for small levels of reward is taken as an indicator of one's sensitivity or resistance to a larger effort/reward balance. However, since less drug is taken later in the session, there are smaller differences in the total amount of drug taken in higher or lower responders, perhaps allowing one to measure biochemical differences after the intake session related more to motivation for substance with less contamination by differences in intake levels (e.g. see Pantazis et al., 2019). An additional possibility would be to use activity measures such as photometry to examine orexin cell activity before and after protracted intoxicant intake; if successful, further studies could then compare innate orexin cell activity with specific molecular differences without requiring intoxicant exposure. Despite the many possible caveats, it will be important to identify innate differences, or differences in drug-related neuro-adaptations, that allow Ox1Rs to promote excessive drinking or intake of other substances.

Addiction, negative affect, and orexin receptors

Addiction likely involves stress-related responding, through negative reinforcement (intake to relieve negative affect), compulsivity (intake despite punishing consequences), and other factors (Koob and Volkow, 2010; Koob, 2013; Hopf and Lesscher, 2014). Orexin receptors have been related to promotion of anxiety- and depression-like states, including both Ox1Rs and Ox2Rs, although anti-anxiety findings have also been observed (see Johnson et al., 2012; Summers et al., 2018 for review). Avoiding predator odor involves Ox1Rs (Staples et al., 2014), and inhibiting Ox1Rs reduces several forms of anxiety including panic models and conditioned fear (Bonaventure et al., 2015; 2017; Dustrude et al., 2018), similar to where reducing orexin function can increase resilience to social stress (Grafe et al., 2018). In addition, stimulating orexin cells increases anxiety and locomotion, but only in a social context (Heydendael et al., 2014) (not in home cage), while stimulating orexin receptors on indirect pathway NAc neurons leads to avoidance (Blomeley et al., 2018). Also, the central amygdala (CeA) likely develops critical neuro-adaptations that promote stress-related aspects of addiction, including dependence-induced enhancement of alcohol intake (Koob and Volkow, 2010; Koob, 2013). Thus, a recent study (Schmeichel et al., 2017) compared LgA versus ShA responding for cocaine, and found that SB systemically or within the CeA reduced the elevated LgA cocaine responding but had no effect on ShA intake. Inhibiting CeA Ox1Rs also reduces conditioned fear (Dustrude et al., 2018). In addition, intra-locus coeruleus injection of SB prevents development (Mousavi et al., 2014) and expression (Azizi et al., 2010) of morphine withdrawal symptoms, without effects on morphine reward (Aston-Jones et al., 2010), and orexin levels are also associated with withdrawal distress symptoms in humans with AUD (von der Goltz et al., 2011). Thus, there is strong precedent for the importance of orexin signaling for expression of negative affect.

However, it is also interesting that not all CeA contributions are stress-related, e.g. the recent demonstration that some CeA cells mediate palatable food reward (Hardaway et al., 2019). Importantly, global Ox1R inhibition in a brain region could impact activity of multiple cell populations with opposing or different effects, underscoring the importance of some caution when assigning particular Ox1R functions during addiction as reward/motivated- versus anxiety/stress-related. Careful cell-type and subregional mapping is likely required, e.g. where orexin in the NAc Shell increases “liking” more rostrally, but elicits more disgust/fear when applied more caudally (Castro et al., 2016). Also, as noted above, things may be further complicated by the possibility that addiction involves positive and negative motivations acting in concert. For example, a number of studies using c-fos as a measure of activation have found that medial orexin neurons are preferentially activated by stress, including withdrawal, while lateral orexin neurons are activated more by putative reward (see Harris et al., 2005; Moorman et al., 2016). However, other findings give more divergent results, including where alcohol seeking activates both lateral (putative reward) and medial (putative stress) orexin cells (Moorman et al., 2016). Even more, stress is not a singular construct (Hopf et al., 2011), e.g. where the impact of stressors may depend on the basal arousal state (cf Blume et al., 2018). Thus, understanding specific positive- and negative-driven intoxicant-related drives may be challenging in the context of addiction, and will require, among other things, careful delineation of orexin’s impact in specific subregions and cell groups of the brain.

Orexin-2-receptors for addiction, negative affect, and reward

While we have focused primarily on Ox1Rs, Ox2Rs also likely play an important role. More generally, Ox2Rs have been considered more important for arousal, e.g. where Ox2Rs but not Ox1Rs contribute to arousal from sleep (Li et al., 2018). However, Ox2Rs, especially in particular brain regions, can also play an important role in expression and regulation of addiction and negative affect (Mahler et al., 2012; James et al., 2017a; Summers et al., 2018). Indeed, one significant shortcoming in the present literature is the greater focus on Ox1Rs and a relative paucity of studies of Ox2R related signaling for addiction, although important work has shown Ox2R contributions within PVT, NAc, mPFC and other areas (see below). In addition, the widely used SB has only an about 40-fold specificity for Ox1Rs over Ox2Rs (Scammell and Winrow, 2011), making it important to show that any SB impact on behavior is not also observed with a more selective Ox2R blocker, e.g. where we find that SB but not an Ox2R blocker in the NAc Shell reduces excessive alcohol binging in mice (Lei et al., 2016b).

Ox2Rs mediate cued reinstatement for nicotine but not self-administration (Uslaner et al., 2014), suggesting importance for relapse and conditioned effects during abstinence rather than primary reward and intake per se. Ox2Rs are also implicated in opiate-related behavior, where LgA escalates heroin intake relative to ShA (similar to cocaine), and increases Ox2R mRNA levels in CeA, while systemic Ox2R inhibition reduces LgA but not ShA responding for heroin (Schmeichel et al., 2015). Some studies also implicate Ox2Rs for alcohol intake, especially in specific brain regions. We find that Ox1Rs but not Ox2Rs in the mouse NAc Shell are important for driving alcohol binging (Lei et al., 2016b), while Ox2Rs in rat NAc Core but not Shell drive lever pressing for alcohol, without impacting sucrose self-administration or locomotor activity (Brown et al., 2013). However, NAc Core Ox2R block also does not reduce cued reinstatement (Brown et al., 2013), which is different from Ox1Rs which contribute to cued reinstatement for a

variety of substances (Mahler et al., 2012; 2014). Also, NAc Shell likely has Ox2Rs, since inhibiting Shell Ox2Rs or Ox1Rs reduces reinstatement of morphine conditioned place-preference (CPP) (Qi et al., 2013). Finally, one study found that systemic inhibition of Ox2Rs but not Ox1Rs decreases alcohol self-administration in rats and alcohol CPP in mice, without changing saccharin intake and alcohol withdrawal (Shoblock et al., 2011). While this might seem to contradict Ox1R-related enhancement of alcohol drinking, the response requirements in this task were rather simple (fixed-ratio 3), and thus might not require recruitment of Ox1R to increase motivation for responding. In addition, others have reported no Ox1R role in alcohol CPP (Vorhees and Cunningham, 2011). Thus, Ox2Rs also likely play a role in regulating important aspects of addiction-related responding, which may in part depend on brain region in which they are activated.

In this regard, considerable work has examined Ox2R function in the paraventricular thalamus (PVT) in relation to addiction and anxiety (e.g., see Matzeu and Martin-Fardon, 2018; Summers et al., 2018). Orexin infusion in PVT reinstates cocaine seeking after LgA training, which is prevented by Ox2R but not Ox1R blockers (Matzeu et al., 2016; see also Matzeu et al., 2018). Anterior but not posterior PVT orexin (and interacting neuropeptides) promote alcohol intake via Ox2R but not Ox1R, and alcohol drinking increases Ox2R but not Ox1R mRNA in the anterior PVT (Barson et al., 2015; 2017). PVT orexin is also linked to cued alcohol reinstatement (Dayas et al., 2008). More generally, stimulating PVT Ox2Rs and PVT projections to NAc Core increase intake and hunger (Meffre et al., 2019), and activating PVT-NAc Core increase wakefulness (Ren et al., 2018), similar to an Ox2R but not Ox1R role in arousal from sleep after orexin cell stimulation (Li et al., 2018). In contrast, PVT Ox1Rs mediate reward-based feeding and increase NAc dopamine levels and locomotion (Choi et al., 2012), although PVT Ox1R

inhibition does not reduce cued cocaine reinstatement (James et al., 2011). Importantly, Barson and colleagues (2015; 2017) and others have noted functional differences of anterior versus posterior PVT, which may contribute to some apparently mixed findings. PVT orexin has also been implicated in negative processing (see Summers et al., 2018). PVT Ox2Rs but not Ox1Rs are important for expression of conditioned place aversion (CPA) to naloxone-precipitated withdrawal after chronic morphine, but are not needed for CPA acquisition or withdrawal signs (Li et al., 2011). Also, footshock-induced anxiety is mediated by PVT Ox2Rs (Li et al., 2010). However, PVT orexin is not involved in all negative contexts, e.g. with no role in conditioned fear (Dong et al., 2015). In contrast, PVT Ox1Rs, but not Ox2Rs blocked systemically, contribute to nicotine withdrawal signs (Plaza-Zabala et al., 2012). Thus, PVT orexin are implicated in many negative influences but can also mediate positive effects, and act through specific projections (e.g. to the NAc Core) and subregion-specific influences.

Ox1Rs for conditioned responding?

One possibility is that Ox1R-mediated motivation for intoxicants is driven by conditioned, learned responding, rather than the primary reinforcing effects themselves. For alcohol, Ox1Rs promote intake (Moorman et al., 2009; Alcaraz-Iborra et al., 2017; Lei et al., 2019) and also unreinforced seeking (Moorman et al., 2017), and preferentially in higher-responding individuals. For cocaine, Ox1Rs primarily sustain responding for higher motivation states or individuals (see above), but does not impact drug-primed reinstatement or low-effort or -reward types of cocaine intake (see Mahler et al., 2014; James et al., 2017b). Interestingly, SB only reduces demand for cocaine when drug-paired cues are present (Bentzley and Aston-Jones, 2015). This is

an area of great interest for future studies, for a number of reasons. Such findings may imply that different aspects of primary experience make differential contributions in the drive for different intoxicants. For example, bottle-mediated alcohol consumption seems like a simple behavior, but may actually involve aspects of conditioned responding that are not required for a simple version of cocaine intake. Indeed, cocaine has faster onset of intoxication compared with alcohol, while many studies of alcohol find strong initial intake (loading up), where heavy intake occurs before interoceptive effects of alcohol intoxication would become apparent. In possible agreement, Ox1R inhibition is effective against alcohol seeking in the absence of reward (tested via cued reinstatement) in higher-drinking individuals (Moorman et al., 2017), similar to effects on reinstatement of seeking for other intoxicants (Mahler et al., 2012; 2014). Also, mPFC Ox1Rs are important for both binge alcohol drinking (Lei et al., 2016b) and cue potentiation of feeding (Cole et al., 2020). Furthermore, Ox1R inhibition does not alter alcohol CPP (Shoblock et al., 2011; Voorhees and Cunningham, 2011). These findings could suggest an Ox1R role in motivation rather than intake or reward *per se* (see also Mahler et al., 2012; 2014). Further, drug-related stress could also act as a conditioned factor driving intake, in addition to primary destabilization of affective homeostasis. Taken together, the central mechanisms that drive addiction could depend on an interaction of a number of factors, including basal motivation for intoxicant in an individual, intake history, the specifics of a given self-administration context (including stress and other cues), and the intoxicating nature of the particular substance (especially fast or slow action).

Ox1Rs and action control

Studies described above implicate Ox1Rs primarily in higher-motivation or cost/effort-resistant aspects of addiction, with little role in cost-free or more moderate effort responding. Also, Ox1R inhibition (sometimes within particular brain regions) often does not impact responding for natural rewards (although there are mixed findings, see above). Thus, it is of interest to examine whether Ox1Rs do or don't regulate other behaviors not directly involving responding for intoxicant, in particular related to control of action initiation. For example, Ox1R inhibition does not impact responding on a stop-signal reaction time task in rats, a measure of behavioral inhibition and control, suggesting a minimal role for Ox1Rs in regulating response inhibition or attention (at least under the conditions studied), although it does reduce overall motivation to perform the task for food (Wiskerke et al., 2019). In contrast, a study using a different behavioral inhibition measure, the Go/NoGo task for highly palatable food, found that behavioral performance correlated with cFos in medial orexin but not LHA cells (Freeman et al., 2018). In addition, several rodent studies implicate OxR1s in motor impulsivity, but much less so for choice impulsivity (delayed discounting, the ability to wait for a larger reward) (Alcaraz-Iborra et al., 2015; Gentile et al., 2018). SB at 30 mg/kg also does not block a simpler task, pavlovian cue learning (Borgland et al., 2009). Thus, these exciting findings are shedding important light on Ox1Rs in action control more generally, with implications for addiction-related behavior.

Sex differences in orexin signaling

In recent years, the rate of hazardous alcohol drinking in females has risen dramatically (White et al., 2015; Grant et al., 2017), and it is essential to understand possible mechanistic differences across the sexes that promote addiction. Female and male rodents have known differences,

including greater intake of alcohol and compulsivity for cocaine (Becker and Koob, 2016). Thus, it is important to understand whether orexin signaling play a different role in females and males during higher-motivation states. This is addressed at length in Grafe and Bhatnagar (2018) (see also Yeoh et al., 2014).

One robust finding is that orexin levels are higher in females, which likely contributes to exaggerated neuroendocrine and behavioral responses to stress in females (Grafe and Bhatnagar, 2018). However, there seem to be important differences across brain regions. Sex and estrous differences for orexin and orexin receptor are observed in hypothalamus (Silveyra et al., 2010), but not cortical and other subcortical areas (Chen et al., 1999; Silveyra et al., 2010; Lee et al., 2015), or in orexin receptor regulation of morphine mesolimbic activation, sucrose intake, or stress-induced cocaine seeking (Narita et al., 2006; Zhou et al., 2012; Cason and Aston-Jones, 2014). Also, systemic Ox1R blockers reduce 2-bottle choice alcohol intake in both sexes (Moorman et al., 2009; Anderson et al., 2014), while Ox1R inhibition impacts operant-based alcohol intake in male P-rats (Jupp et al., 2011) with only a trend in females (Anderson et al., 2014). Additional studies have examined seeking, which is responding (such as lever pressing) in the absence of reward, in contrast to the self administration in the just-described findings. Inhibiting Ox1Rs disrupts operant-based cocaine or sucrose seeking only in males, even though females have overall similar response levels (Zhou et al., 2012; Cason and Aston-Jones, 2014), and blocking Ox1Rs reduces alcohol seeking in males (Mahler et al., 2012), with effects in females only if alcohol is present (Dhaher et al., 2010). However, Ox1Rs are critical for stress-induced seeking for cocaine in both sexes (Zhou et al., 2012). In addition, quinine-resistant alcohol intake, which is highly dependent on Ox1Rs in males (Lei et al., 2016a), shows no sex differences (Sneddon et al., 2019) or greater quinine-resistance in females (Fulenwider et al., 2019; Radke et

al., 2019), depending on the model. Also, several other perseverative behaviors are overall similar between sexes (Agrati et al., 2005; Tanimura et al., 2008; Mitra et al., 2017), except for cocaine compulsion (Becker and Koob, 2016). Thus, there is a clear precedent for differential orexin regulation of addiction-related behaviors in males and females, but a great deal of work remains.

More generally, interesting recent studies have also shown that females have impaired habituation to cognitive deficits caused by repeated restraint stress, which is mediated through elevated orexin levels (Grafe et al., 2018). In concert, orexin neurons have similar dendritic complexity in males and females in the absence of stress, but repeated stress decreases orexin cell morphology in males but not females (Grafe et al., 2019). Lack of habituation to stress in females could reflect molecular differences in orexin signaling; while not examined for orexin receptors, CRF receptors in midbrain dopamine neurons from females show reduced CRF-mediated internalization relative to males (Valentino and Bangasser, 2016). It is also interesting that reducing orexin function can increase resilience to social stress (Grafe et al., 2018). In addition, Ox1Rs have different impacts on open field anxiety-related behavior depending on age and sex (Blume et al., 2018), and there are sex differences in orexin markers in humans with depression (Lu et al., 2017). Thus, it will be very important to better understand how orexin receptors might differently impact addiction and anxiety behaviors in females and males, including the possibility of differential adaptations or receptor-signaling pathways, especially as the level of challenge and negative affect rises. These findings also validate the value of studying blood orexin levels in humans (Lu et al., 2017) and rats as a potential biomarker for at-risk states.

Addictants can alter the number of Orexin Neurons

Studies described above have implicated greater Ox1R signaling under conditions with higher motivation, including both intake-dependent and individual differences, and the underlying molecular adaptations that mediate such greater motivation are of great interest. Recent work has uncovered the very interesting possibility that stronger addiction is associated with increased number of LHA orexin cells. In particular, access to cocaine under InA (relative to LgA or ShA, see above) leads to development addiction-related behaviors, including greater cost-resistance, reinstatement, and negative mood (James et al., 2019a; 2019b), and also produces a significant increase in the number of LHA orexin neurons. Increased orexin neurons is still observed after 150 days of abstinence, and is associated with greater reinstatement after abstinence (incubation). In addition, lower doses of Ox1R blocker (SB 10 mg/kg) reduce drug-related behaviors after InA, while higher doses (SB 30 mg/kg) were needed in other exposure groups. Related studies also indicate greater number of orexin cells in individuals with higher motivation for cocaine, and, interestingly, that cocaine demand correlates with the number of orexin cells remaining after orexin knockdown (Pantazis et al., 2019). Together, these studies indicate that conditions leading to higher cocaine addiction increase the number of LHA orexin cells and enhance Ox1R regulation of addiction-related behavior. In addition, increased numbers of orexin-expressing neurons have been observed both in heroin-using humans and morphine-exposed mice (Thannickal et al. 2018), suggesting that changes in orexin cell levels may occur across addictant types. It is also important to note that most measures of increased numbers of neurons use immunohistochemistry, so increased numbers could just be upregulation of peptide expression in normally low-expressing neurons.

Results for alcohol and orexin neurons are more mixed. In agreement with cocaine, an increased area of orexin mRNA is observed after protracted alcohol intake in alcohol-preferring

rats (Lawrence et al., 2006). However, binge alcohol intake in C57 mice decreases the number of cells with orexin immunoreactivity in the lateral hypothalamus (Olney et al., 2015), although with no change in preproorexin mRNA (Olney et al., 2017). Olney et al. (2015) also find that sucrose drinking in mice also decreases orexin levels, similar to alcohol, and that both alcohol and sucrose intake are reduced by systemic SB. Whether these mixed findings reflect species differences or other aspects of alcohol exposure (lever pressing versus simple bottle intake) represents interesting and important avenues for future research.

Therapy

Given the relation between Ox1Rs and high-motivation responding for addictive substances, Ox1R (and perhaps Ox2R) antagonists may represent a valuable pharmacological intervention in addicted humans (Khoo and Brown, 2014; Li et al., 2016; James et al., 2017b; Walker and Lawrence, 2017; Perry and Zhang, 2018). Belsomra (suvorexant) is a dual orexin receptor antagonist (DORA) used in humans for insomnia, and would be a poor complement with alcohol addiction (mixing two sedatives). Since Ox2Rs may be more linked to sleep regulation, use of blockers specific to Ox1Rs would alleviate such concerns. However, the observation that lower doses of orexin receptor blockers are effective against compulsion-like aspects of alcohol addiction (Lei et al., 2016a), which is a key contributor to the expression of addiction (see above), might suggest that lower doses of suvorexant could be used in humans to decrease compulsive aspects of responding for alcohol in humans while minimizing possible side effects. Also, there are perhaps less sedative concerns with cocaine, and a recent study found preliminary evidence for efficacy of suvorexant to reduce cocaine relapse related states in human addicts (Suchting et al.,

2019). One additional potential concern is that repeated SB can alter subsequent response to SB, which would complicate drug dosing (Zhou et al., 2012). Finally, hypoarousal and lower orexin levels have been linked to depression (James et al., 2017a). Thus, use of orexin receptor blockers to treat addiction should involve careful monitoring for depressed mood (which in itself can be a risk factor for some aspects of addiction).

Interestingly, a recent study described a different method to influence orexin function: cocaine increases excitatory drive onto LHA orexin neurons, and metabotropic glutamate receptor (mGluR) agonists decrease both cocaine responding and glutamatergic drive on orexin cells (Yeoh et al., 2019). Thus, both orexin receptor drugs and orexin modulators could represent powerful interventions to counteract the strong motivation and negative affect that orexins can promote. Recent findings have also identified human Ox1R genetic variants linked to aggression and anxiety (Harro et al., 2019). Thus, orexin signaling remains an area of promising intervention for some human neuropsychiatric conditions, including pathologically elevated motivation, with much additional work needed to understand and successfully intervene in these pathologies.

Summary

Taken together, many lines of evidence support the idea that orexin receptor signaling contributes strongly for higher-salience addiction-related states. This includes responding with more motivating rewards and/or greater response requirements, individual differences in motivation and drive, and anxiety and stress related contributions. While the molecular bases of such differences remain unclear, differences in the number of orexin-expressing cells are an interesting mechanism observed across several intoxicants, although much remains to be learned

about adaptations and individual differences in other orexin related signaling molecules. In addition, the orexin literature has tended to examine a more limited set of brain regions, e.g. where orexin receptors in the cortex have only a few studies in a limited number behavioral tasks, leaving an incomplete picture of the overall importance of cortical orexin. Studies have also focused more on Ox1Rs, although more recent studies have uncovered the important role of Ox2Rs. In addition, there are important differences in orexin signaling in females and males, which has important implications for sex-specific therapeutic interventions. Thus, while the extant literature provides a compelling framework suggesting that orexin receptors are particularly important for higher-motivation states during addiction-related behaviors, there is still much to learn about the importance of signaling in particular brain regions, whether this signaling is acting through positive or negative valence effects, and the critical differences in orexin function in females and males.

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Figure 1. Orexin receptor contributions in specific brain areas to particular addiction-related behaviors. Cartoon of studies mentioned in the manuscript where orexin receptor

contributions within specific brain areas were examined. LC: Locus Coeruleus, WD: withdrawal; other acronyms defined in the main text.