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# Orexin/hypocretin system: Role in food and drug overconsumption

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## Abstract

The neuropeptide orexin/hypocretin (OX), while largely transcribed within the hypothalamus, is released throughout the brain to affect complex behaviors. Primarily through the hypothalamus itself, OX homeostatically regulates adaptive behaviors needed for survival, including food intake, sleep-wake regulation, mating, and maternal behavior. However, through extra-hypothalamic limbic brain regions, OX promotes seeking and intake of rewarding substances of abuse, like palatable food, alcohol, nicotine, and cocaine. This neuropeptide, in turn, is stimulated by the intake of or early life exposure to these substances, forming a non-homeostatic, positive feedback loop. The OX receptor involved in these behaviors, adaptive behavior or substance seeking and intake, is dependent on the particular brain region that contributes to them. Thus, we propose that, while the primary function of OX is to maintain arousal for the performance of adaptive behaviors, this neuropeptide system is readily coopted by rewarding substances that involve positive feedback, ultimately promoting their abuse.

### Keywords

sleep-wake; mating; maternal behavior; sucrose; fat; alcohol; nicotine; cocaine; seeking; intake

## 1. Introduction

In 1998, the neuropeptide orexin/hypocretin (OX) was identified independently by two different laboratories, both of which suggested that it played a major role in food intake and energy homeostasis (de Lecea et al., 1998; Sakurai et al., 1998). Although the cell bodies that transcribe this neuropeptide were shown to be largely restricted to the hypothalamus that regulates food intake, these neurons were also found to send extensive projections throughout the brain, most notably to the limbic system (Peyron et al., 1998; Trivedi, Yu, MacNeil, Van der Ploeg, & Guan, 1998). Numerous studies over the past 20 years have demonstrated that hypothalamic OX neurons and their projections have broader functions beyond homeostatic food intake, controlling a range of other adaptive behaviors such as sleep-wake regulation, mating, and maternal behavior. Of particular interest is the additional

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evidence that OX promotes dysregulated, non-homeostatic intake, such as occurs with abused, rewarding substances. It stimulates excessive intake of palatable sweet and fatty foods, alcohol, nicotine, and cocaine among other substances, and in many cases, it is in turn stimulated by their intake as part of a positive feedback loop. This is the focus of the present review.

## 2. Anatomy of orexin/hypocretin-expressing neurons

The first description of OX came from de Lecea and colleagues (1998), who identified a gene which they termed hypocretin, given its nearly exclusive expression in the *hypo*thalamus and its similar amino acid sequence to the gut hormone se*cretin*. These authors noted that this precursor gene actually encodes two peptides, which they termed hypocretin 1 and hypocretin 2 (de Lecea et al., 1998). Soon thereafter, Sakurai and colleagues (1998) identified the same 130 amino acid neuropeptide gene, which they termed orexin, after the Greek word for appetite, *orexis*, and they noted that its sequence is well-conserved between rats, mice, and humans. This group also identified the same encoded peptides, orexin-A (OX-A), equivalent to hypocretin 1 and composed of 33 amino acids, and orexin-B (OX-B), equivalent to hypocretin 2 and composed of 28 amino acids, and they noted that the two peptides were 46% identical in their sequence (Sakurai et al., 1998). A remarkable feature of OX is that, despite its restricted expression in a discrete set of predominantly hypothalamic nuclei, the neurons expressing this neuropeptide project widely to brain regions involved in feeding, arousal, and hormone release (Peyron et al., 1998; Trivedi et al., 1998) to influence an array of behaviors. This section will describe the anatomical locations of the OX neurons, their efferent and afferent projections, and the neurochemical properties of the OX receptors.

### 2.1. Cell bodies

Neurons transcribing OX comprise a small cluster of neurons that lie predominantly within the hypothalamus (Peyron et al., 1998; Sakurai et al., 1998). The subnuclei in which they have been described differ somewhat across publications, depending on the anatomical terminology used by the particular laboratory. In the rat, about half of the 6700 OX-expressing neurons can be found in the perifornical region of the hypothalamus, with the rest found across the lateral hypothalamus, dorsomedial hypothalamus, and posterior hypothalamus, as well as the subthalamus (de Lecea et al., 1998; Modirrousta, Mainville, & Jones, 2005; Peyron et al., 1998; Sakurai et al., 1998). While this distribution of OX neurons overlaps significantly with that of another orexigenic neuropeptide, melanin-concentrating hormone, these two neuropeptides show virtually no co-localization within neurons (Hakansson, de Lecea, Sutcliffe, Yanagisawa, & Meister, 1999). Orexin/hypocretin-expressing neurons have been identified in a host of mammalian vertebrates, including rats, mice, and humans (de Lecea et al., 1998; Sakurai et al., 1998) as well as dogs (Lin et al., 1999), sheep (Archer, Findlay, Rhind, Mercer, & Adam, 2002), pigs (Dyer, Touchette, Carroll, Allee, & Matteri, 1999), and non-human primates (Horvath, Diano, & van den Pol, 1999). They have also been observed in non-mammalian vertebrates, such as zebrafish, where OX neurons lie in the rostral hypothalamus and preoptic area (Kaslin, Nystedt, Ostergard, Peitsaro, & Panula, 2004).

While not with melanin-concentrating hormone, OX is found to co-localize with a number of other neuropeptides and neurotransmitters. Most notably, 94% of OX neurons also contain the opioid neuropeptide dynorphin (Chou et al., 2001), and both OX and dynorphin have even been observed within the same synaptic vesicles (Muschamp et al., 2014). There is also significant co-localization of OX with glutamate (Rosin, Weston, Sevigny, Stornetta, & Guyenet, 2003; Torrealba, Yanagisawa, & Saper, 2003) and prolactin (Risold, Griffond, Kilduff, Sutcliffe, & Fellmann, 1999) and a more limited co-localization with galanin (Hakansson et al., 1999), nociceptin/orphanin FQ (Maolood & Meister, 2010), neurotensin (Furutani et al., 2013), and nitric oxide (Cheng, Kuchiiwa, Gao, Kuchiiwa, & Nakagawa, 2003). Thus, the release of OX is likely to co-occur with these other neurochemicals, which may act on different timescales and have different effects from OX.

### 2.2. Receptors

The two receptors for OX have different binding affinities for the OX peptides. The orexin 1 receptor (OX1R, also called hypocretin receptor 1) primarily interacts with OX-A, requiring for a half-maximum response a concentration of 30 nM of OX-A but 2500 nM of OX-B (Sakurai et al., 1998). This is in contrast to the orexin 2 receptor (OX2R, also called hypocretin receptor 2), which interacts nearly equally with OX-A and OX-B, requiring for a half-maximum response a concentration of 34 nM of OX-A and 60 nM of OX-B (Sakurai et al., 1998). Thus, OX-A binds to both OX1R and OX2R with equal affinity, while OX-B binds almost exclusively to OX2R. Both receptors interact with Gq, Gs, and Gi proteins (Magga et al., 2006; Tang et al., 2008) and have been observed both pre- and post-synaptically (van den Pol, Gao, Obrietan, Kilduff, & Belousov, 1998).

### 2.3 Efferent projections

While the OX peptides are released widely through the brain, their projections are most highly concentrated in different limbic regions involved in arousal and emotion. Thus, OX axons are particularly dense within the hypothalamus from where they follow both ascending and descending pathways (Peyron et al., 1998). The heaviest OX projection is to the locus coeruleus, but axons are also very dense in multiple regions in the forebrain (bed nucleus of the stria terminalis, paraventricular and central medial thalamus, and subthalamus), hindbrain (substantia nigra, nucleus of the solitary tract, periaqueductal gray, dorsal raphe and raphe magus, and reticular nuclei), and posterior hypothalamus (Peyron et al., 1998). The OX receptors are also highly expressed in each of these regions, where in many cases only one or the other receptor is present. For example, the locus coeruleus and bed nucleus have heavy gene expression of the OX1R but no detectable OX2R, similar to the hypothalamic ventromedial nucleus which contains OX1R but not OX2R; in contrast, the central medial thalamus contains OX2R but not OX1R, and the hypothalamic paraventricular nucleus has dense expression of OX2R but not OX1R (Trivedi et al., 1998). Nuclei containing both receptors at similar levels include the paraventricular thalamus, dorsal raphe, and ventral tegmental area (Marcus et al., 2001; Trivedi et al., 1998). It is notable that brain regions not related to arousal or emotion contain fewer, if any, OX fibers and receptors, including the caudate putamen, pretectal area, and oculomotor nucleus (Peyron et al., 1998; Trivedi et al., 1998).

### 2.3. Afferent inputs

In line with their projections to limbic brain regions involved in arousal and emotion, the OX cell bodies also receive afferent input from brain regions and neurochemicals involved in these behavioral functions, with the connections often being reciprocal. The heaviest input to OX neurons comes from the lateral septum and medial preoptic area as well as the bed nucleus of the stria terminalis and posterior hypothalamus to which these neurons themselves project (K. Yoshida, McCormack, Espana, Crocker, & Scammell, 2006). In addition, the neurons receive input from select nuclei of the forebrain (infralimbic cortex, cingulate cortex, nucleus accumbens, and amygdala), hindbrain (locus coeruleus, ventral tegmental area, substantia nigra, periaqueductal gray, and dorsal raphe), and hypothalamus (suprachiasmatic nucleus, anterior hypothalamus, ventromedial hypothalamus, and supramammillary nucleus), but not from regions such as the caudate putamen, somatosensory cortex, or piriform cortex that are unrelated to arousal and emotion (K. Yoshida et al., 2006). Consistent with these diverse afferents, the OX neurons are found to express different types of receptors, in addition to their own, the OX1R (Backberg, Hervieu, Wilson, & Meister, 2002) and OX2R (Yamanaka, Tabuchi, Tsunematsu, Fukazawa, & Tominaga, 2010). These other receptors include those for serotonin (Collin, Backberg, Onnestam, & Meister, 2002), acetylcholine (Chou, Rotman, & Saper, 2004; Garcia et al., 2015), adenosine (Thakkar, Winston, & McCarley, 2002), GABA (Backberg, Collin, Ovesjo, & Meister, 2003; Backberg, Ultenius, Fritschy, & Meister, 2004), and leptin (Hakansson et al., 1999), although rarely dopamine (Bubser et al., 2005), and also those for the neuropeptides, cholecystokinin (Tsujino et al., 2005), corticotropin-releasing factor (Winsky-Sommerer et al., 2004), neurotensin (Furutani et al., 2013), neuropeptide Y (Fu, Acuna-Goycolea, & van den Pol, 2004), and galanin-like peptide (Kageyama et al., 2006), as well as the mu opioid receptor (Y. Zhou et al., 2006). Together, these connections position OX at the center of a network regulating motivation.

## 3. Role of orexin/hypocretin in homeostatic feeding and other adaptive behaviors

While OX was originally noted for its role in food intake and energy homeostasis, it soon became apparent that this neuropeptide participates in a host of other behaviors, many of which serve to promote survival. These include not only homeostatic food intake, but also sleep-wake regulation, mating behavior, and maternal behavior.

### 3.1. Homeostatic food intake

Studies provide clear evidence that OX neurons act to promote food seeking behavior, as normally occurs during a prolonged fast, restricted feeding, and hypoglycemia, with the activation of these neurons being reduced once food is consumed. Gene expression and brain peptide levels of OX have been reported to be elevated after a 48 or 72 hour fast (Fujiki et al., 2001; Moriguchi, Sakurai, Nambu, Yanagisawa, & Goto, 1999; Sakurai et al., 1998). Similarly, under a restricted feeding paradigm, OX neurons are more active immediately prior to scheduled food availability (Johnstone, Fong, & Leng, 2006; Mieda et al., 2004), while mice lacking the OX gene show reduced waking and locomotor activity during the

food-anticipatory period (Akiyama et al., 2004; Mieda et al., 2004). Gene expression and peptide levels as well as the activity of OX neurons are also increased by a state of hypoglycemia (Bayer et al., 2000; Griffond, Risold, Jacquemard, Colard, & Fellmann, 1999), which is corrected when glucose levels are restored by food intake (Cai et al., 2001). Although hyperglycemia does not appear to affect mRNA levels of OX (Griffond et al., 1999), glucose at physiological levels reduces the firing of OX neurons in a concentration-dependent manner (Burdakov, Gerasimenko, & Verkhratsky, 2005). This indicates that under physiological conditions, OX neurons are activated and transcribe the neuropeptide when glucose levels are low, such as from fasting or restricted feeding, and they are inhibited when glucose levels are high.

The role of OX in stimulating intake of standard food appears to involve the OX1R primarily in the hypothalamic areas where the OX neurons exist, with OX likely acting on these neurons as well as on other local neuronal populations. Most notably, the intake of laboratory chow is increased by pharmacogenetic activation of OX neurons and reduced by extensive ablation of these neurons or peripheral injection of an OX1R antagonist (Haynes et al., 2000; Inutsuka et al., 2014). Through microinjection experiments, these effects of OX in promoting food intake are found to be largely due to the action of OX-A in the same hypothalamic subregions where OX itself is transcribed, namely, the perifornical area, lateral hypothalamus, and dorsomedial hypothalamus, with OX-B having little effect on feeding (Dube, Kalra, & Kalra, 1999; Sweet, Levine, Billington, & Kotz, 1999). These actions of OX-A are thus likely be mediated by OX1R, which are located both on OX neurons themselves as well as on neurons expressing the feeding-promoting neuropeptide, melanin-concentrating hormone (Backberg et al., 2002). In contrast, there is little effect on food intake from OX injected in other brain areas, including the ventromedial hypothalamus, preoptic area, amygdala, ventral tegmental area, or nucleus of the solitary tract (Dube et al., 1999; Sweet et al., 1999). Thus, when promoting homeostatic food intake, OX appears to act predominantly on OX1R in the dorsal and lateral regions of hypothalamus, where it may be responding to physiological signals such as a drop in glucose levels that occurs when animals most need to seek out and consume food.

### 3.2. Sleep-wake regulation

Feeding and waking are closely associated with one another. During food deprivation, animals show shorter episodes of sleep, which decrease in frequency as the length of deprivation increases (Borbely, 1977; Dewasmes, Duchamp, & Minaire, 1989). They also exhibit enhanced locomotor activity (Jones, Bellingham, & Ward, 1990; Mabry & Campbell, 1975; Moskowitz, 1959), possibly reflecting an increase in foraging behavior. Similar to food seeking, OX is robustly tied to waking behavior in the sleep-wake cycle, with this behavior caused in part by its release in the locus coeruleus. This connection has been particularly noted in studies of narcolepsy, which in canines is caused by a disruption of the OX2R (Lin et al., 1999) and in humans is associated with nearly undetectable numbers of OX neurons in the brain and levels of OX in cerebrospinal fluid (Nishino, Ripley, Overeem, Lammers, & Mignot, 2000; Thannickal et al., 2000). Moreover, narcolepsy is ameliorated by intranasal administration of OX-A (Baier et al., 2011). In line with these findings, manipulations of endogenous OX gene expression affect waking and sleep. Mice lacking the

OX gene show behavioral state instability, having frequent and rapid transitions between wake and sleep periods (Chemelli et al., 1999; Diniz Behn, Klerman, Mochizuki, Lin, & Scammell, 2010; Mochizuki et al., 2004), and zebrafish overexpressing OX show increased arousal and reduced initiation and duration of rest periods (Prober, Rihel, Onah, Sung, & Schier, 2006). Under normal conditions, the firing of OX neurons is closely tied to the state of arousal, occurring most frequently during active waking and less frequently during quiet waking, and it is absent during slow wave sleep, is transient during rapid eye movement sleep, and increases again just prior to waking (Lee, Hassani, & Jones, 2005; Takahashi, Lin, & Sakai, 2008). Levels of OX peptide in the hypothalamus and thalamus have also been found to be higher during waking compared to sleep (Kiyashchenko et al., 2002; Y. Yoshida et al., 2001). Perhaps the most compelling evidence that OX firing promotes waking is the finding that optogenetic stimulation of OX neurons increases the probability of a transition from sleep to wakefulness (Adamantidis, Zhang, Aravanis, Deisseroth, & de Lecea, 2007), and this appears to be largely due to changes in the activity of OX1R in the locus coeruleus (Carter et al., 2012; Hasegawa, Yanagisawa, Sakurai, & Mieda, 2014) where OX afferents are most dense (Peyron et al., 1998). Collectively, these studies confirm that OX, through both OX1R and OX2R, stimulates an aroused state, which is conducive to seeking for food.

### 3.3. Mating behavior

Orexin/hypocretin has also been tied to mating behavior, with published studies examining male rats and suggesting that this occurs largely through OX1R in the hypothalamic medial preoptic area. Neurons expressing OX are activated during copulatory behavior (Muschamp, Dominguez, Sato, Shen, & Hull, 2007), and injection of OX-A into the medial preoptic area increases sexual arousal and performance (Gulia, Mallick, & Kumar, 2003) while systemic blockade of the OX1R impairs copulatory behavior (Muschamp et al., 2007). These effects may also be due, in part, to an effect on the rewarding or motivational aspects of this behavior. This is suggested by evidence that lesions of OX neurons prevent the formation of conditioned place preference for a chamber previously paired with sexual behavior (Di Sebastiano, Wilson-Perez, Lehman, & Coolen, 2011) and that OX fibers are apposed to tyrosine hydroxylase-positive neurons in the ventral tegmental area that also show increased activation during copulation (Muschamp et al., 2007). Thus, mating is another behavior, related to arousal and survival, which is enhanced by the activity of OX, with the behavior itself affected by OX acting on OX1R in the hypothalamic medial preoptic area and its reward predominantly involving OX in the limbic ventral tegmental area.

### 3.4. Maternal behavior

Studies also indicate that OX enhances maternal behavior, occurring as with mating behavior through OX1R in the hypothalamic medial preoptic area. Gene expression of OX is increased in pregnant and lactating female rats compared to nonpregnant or nonlactating rats (Kanenishi et al., 2004; Sun, Narita, Murata, Honda, & Higuchi, 2003), and the number of OX-expressing neurons is also increased during lactation (Sun et al., 2003). In accord with this, injection of OX-A into the cerebral ventricles or medial preoptic area specifically increases licking and grooming of pups but not of the self, while peripheral or medial preoptic area injections of an OX1R antagonist reduces these behaviors (D'Anna & Gammie, 2006; Rivas, Torterolo, Ferreira, & Benedetto, 2016). It is possible that the

increase in OX during the perinatal period also contributes to other behaviors observed in mothers during this time, including disturbed sleep (Bei, Coo, & Trinder, 2015) and increased caloric intake (Whichelow, 1975), which are ultimately tied to care of the offspring.

## 4. Role of orexin/hypocretin in non-homeostatic intake

Whereas adaptive behaviors are generally performed in a homeostatic manner that involves negative feedback, the intake of rewarding substances of abuse is often non-homeostatic, involving a positive feedback loop that promotes overconsumption. While the mechanisms behind these adaptive and overconsumption behaviors may seem distinct, the behaviors are known to share in common their functioning through dopamine in the nucleus accumbens shell. Levels and release of accumbal dopamine are increased in response to adaptive behaviors, including food intake (Martel & Fantino, 1996), the waking state (Lena et al., 2005), mating behavior (Pfaus et al., 1990), and maternal behavior (Champagne et al., 2004), and also prior to these behaviors (Champagne et al., 2004), with dopamine seemingly contributing to their initiation (Pisanu et al., 2015). Similarly, accumbal dopamine levels are increased by the intake of rewarding substances, including palatable foods high in fat or sugar (Rada, Avena, Barson, Hoebel, & Leibowitz, 2012; Rada, Avena, & Hoebel, 2005; Sahr et al., 2008) and drugs of abuse such as alcohol (Howard, Schier, Wetzel, Duvauchelle, & Gonzales, 2008), nicotine (Cadoni & Di Chiara, 2000; Pontieri, Tanda, Orzi, & Di Chiara, 1996), and cocaine (Pontieri, Tanda, & Di Chiara, 1995), and they are also elevated prior to the initiation of their intake (Cacciapaglia, Saddoris, Wightman, & Carelli, 2012; Doyon et al., 2003; Suto, Ecke, You, & Wise, 2010). Notably, the increase in accumbal dopamine produced by the intake of these rewarding substances is significantly greater than that induced by the performance of adaptive behaviors (Martel & Fantino, 1996), suggesting that the system governing these behaviors differs only in degree. While OX is similar to dopamine in having an important function in promoting both the adaptive behaviors and non-homeostatic consumption of the rewarding substances, it is notable that OX acts through the hypothalamus to exert its effects on the homeostatic behaviors but acts through the extra-hypothalamic limbic regions to exert its effects on the non-homeostatic behaviors. Thus, OX increases the intake, willingness to work, seeking behavior, and the reward derived from the intake of rewarding substances, by acting through the nucleus accumbens, ventral tegmental area, and paraventricular nucleus of the thalamus, where local application of OX leads to increased extracellular levels of accumbal dopamine (Choi et al., 2012; Korotkova, Sergeeva, Eriksson, Haas, & Brown, 2003; Narita et al., 2006; Patyal, Woo, & Borgland, 2012). By working primarily outside of the hypothalamus to affect the use of rewarding substances, the role of OX in non-homeostatic behaviors may be fundamentally different from that of homeostatic behaviors geared toward survival, promoting excess consumption that can lead to addiction.

### 4.1. Role of orexin/hypocretin in overeating

In stimulating the intake of standard food to maintain physiological glucose levels, OX acts in a homeostatic manner. This differs greatly from the role of OX in the consumption of palatable foods high in fat or sugar, as OX is found not only to promote their intake but also

to be significantly enhanced by their intake. This interaction between OX and palatable food reflects a positive feedback loop that, with a stronger OX signal occurring after each intake episode, may contribute to the overconsumption of fat and sugar.

**4.1.1. Effects of orexin/hypocretin on aspects of palatable food intake—**Similar to its role in controlling intake of standard food, endogenous OX appears to promote the consumption of palatable foods primarily through its actions on OX1R. Mice lacking the OX gene, compared to wild-type mice, consume less sucrose when available *ad libitum*, even after accounting for differences in their locomotor activity (Matsuo et al., 2011). A role for the OX1R in mediating intake of palatable food is supported by the findings that systemic injection of the OX1R antagonist, SB-334867, reduces operant responding for both sucrose and saccharin pellets, in a minimal work paradigm with a fixed ratio 1 schedule (Cason & Aston-Jones, 2013a, 2013b, 2014). This antagonist also suppresses binge-like intake of sucrose, saccharin, and fructose solutions under an intermittent-access home cage drinking paradigm (Alcaraz-Iborra, Carvajal, Lerma-Cabrera, Valor, & Cubero, 2014; Rorabaugh, Stratford, & Zahniser, 2014) and reduces intake of a high-fat diet under both *ad libitum* and limited-access feeding paradigms (Choi, Davis, Fitzgerald, & Benoit, 2010; Valdivia, Patrone, Reynaldo, & Perello, 2014; White et al., 2005). Other OX1R antagonists similarly reduce intake of sweet-fat foods whether under *ad libitum* or intermittent-access feeding paradigms (Piccoli et al., 2012; Steiner, Sciarretta, Pasquali, & Jenck, 2013). The one study of an OX2R antagonist, showing no effect of systemic injection of JNJ-10397049 on the consumption of Nutella® (Piccoli et al., 2012), provides support for the specific role of the OX1R but not OX2R in palatable food intake.

In contrast to the primary role of the hypothalamus in mediating OX's effect on intake of standard food, multiple extra-hypothalamic limbic brain areas, including the nucleus accumbens shell, ventral tegmental area, and paraventricular nucleus of the thalamus, are suggested by central injection studies to be involved in OX's stimulatory effect on intake of palatable food. Thus, OX-A increases the intake of M&Ms® when injected into the nucleus accumbens shell (Castro, Terry, & Berridge, 2016), of a sucrose solution and high-fat diet after injection into the ventral tegmental area (Terrill et al., 2016), and of a sucrose solution after injection into the paraventricular thalamus (Barson, Ho, & Leibowitz, 2015). Conversely, the OX1R antagonist SB-334867 reduces drinking of sucrose when injected into the ventral tegmental area (Terrill et al., 2016), and knockdown of the OX1R in the paraventricular thalamus suppresses the consumption of a high-fat diet (Choi et al., 2012). A possible additional role for hypothalamic and hindbrain nuclei in the effects of OX is suggested by findings that high-fat diet consumption is stimulated by injection of OX-A into the third ventricle in the area of the hypothalamus (Clegg, Air, Woods, & Seeley, 2002) and also the fourth ventricle (Kay, Parise, Lilly, & Williams, 2014) and dorsal vagal complex (Zheng, Patterson, & Berthoud, 2005). Thus, this evidence suggests that homeostatic intake of palatable foods is controlled by OX acting in the hypothalamus, while non-homeostatic intake is controlled by OX in extra-hypothalamic limbic brain areas.

In addition to intake of palatable food, OX is also found to increase an individual's willingness to work for this food, as measured using a progressive ratio task that successively increases the number of operant responses required to earn a reinforcer, with

the breakpoint being the requirement at which responding ceases. The involvement of endogenous OX in the performance of this task is suggested by the findings that ventricular injection of OX-A increases the breakpoint for sucrose (Choi et al., 2010; Kay et al., 2014), while peripheral injection of the OX1R antagonist SB-334867 reduces the breakpoint for sucrose (Cason & Aston-Jones, 2013b; Espana et al., 2010), high-fat diet (Borgland et al., 2009), and sweet-fat pellets (Choi et al., 2010). The specific brain site involved in this response has yet to be identified, with OX-A in the ventral tegmental area failing to affect the breakpoint for sucrose (Terrill et al., 2016) and knockdown of the OX1R in the paraventricular thalamus failing to affect breakpoint for high-fat diet (Choi et al., 2012). Given its strong ties with motivation (Hamid et al., 2016), the nucleus accumbens is likely to be the brain region that mediates the effect of OX on the willingness to work for palatable food.

The possibility that OX also affects the seeking of palatable food, measured by reinstatement following extinction, is suggested by some but not all reports. The OX1R antagonist SB-334867 at 30 mg/kg has been shown to inhibit cue-induced reinstatement of responding for sucrose or saccharin in male rats (Cason & Aston-Jones, 2013a, 2013b). This effect of the antagonist, however, is not evident in female rats (Cason & Aston-Jones, 2014), nor is it seen at a lower dose (10 mg/kg) in male rats with cue-induced reinstatement for sweetened condensed milk (Martin-Fardon & Weiss, 2014a). Although results clearly show that OX acts through the OX1R to promote the intake of a range of palatable foods and the willingness to work for these foods, the possibility that it affects the seeking for these foods requires further investigation.

**4.1.2. Effects of palatable food intake on orexin/hypocretin—**In addition to inducing palatable food intake, endogenous OX itself is found to be highly responsive to the consumption of palatable food, with both its gene expression and peptide levels affected in a time-dependent manner. While the expression of OX is initially reduced during the first 30 minutes after consumption of sucrose or non-caloric saccharin compared to water (Alcaraz-Iborra et al., 2014), similar to the homeostatic inhibition of OX neuron firing induced by a physiological rise in glucose (Burdakov et al., 2005), further measurements indicate that OX peptide levels are subsequently increased by intake of palatable food. They are elevated one hour after the drinking of saccharin compared to water, to a level higher than after standard laboratory chow (Furudono, Ando, Yamamoto, Kobashi, & Yamamoto, 2006), and also two-to-four hours after the eating of sucrose pellets compared to chow (Olszewski et al., 2009). Similarly, endogenous OX is stimulated by the consumption of palatable diets high in fat content. Gene expression of OX is elevated at two hours after a high-fat compared to low-fat meal (Gaysinskaya, Karatayev, Chang, & Leibowitz, 2007), and OX mRNA and the number of OX-A-immunoreactive neurons are higher after three weeks of *ad libitum* high-fat diet consumption (Park et al., 2004; Wortley, Chang, Davydova, & Leibowitz, 2003). It is notable that this increase in OX mRNA and peptide level seen during the first three weeks of high-fat diet access is no longer evident after eight or twelve weeks of access (Cai et al., 1999; Nobunaga et al., 2014; Xu, Wan, Tang, Wu, & Cai, 2008), similar to the temporal pattern of food intake on a high-fat diet with hyperphagia occurring during the first few weeks of access but not later (Honors, Hargrave, & Kinzig, 2012; Vasselli & Maggio, 1990; J. Wang

et al., 1998). These findings with foods rich in sugar and fat support the idea that OX functions in a positive feedback loop with palatable food, with OX acting at the OX1R to both promote and ultimately be increased by their intake, resulting in their overconsumption.

#### 4.1.3. Effects of prenatal exposure to palatable food on orexin/hypocretin—

Similar to adult intake, exposure to palatable food during the prenatal period can affect endogenous OX expression and levels. Rats exposed *in utero* to a high-fat diet compared to a balanced diet show in adolescence and adulthood higher gene expression and peptide levels of OX (Chang, Gaysinskaya, Karatayev, & Leibowitz, 2008; Poon, Barson, Fagan, & Leibowitz, 2012). This increase in endogenous OX in prenatally-exposed animals is accompanied by an increase in the intake of calories of standard chow and also by a higher preference for the high-fat diet (Chang et al., 2008). With endogenous OX in adults shown to promote intake of a palatable fat-rich diet, this finding suggests that increased release of OX contributes to the excessive consummatory behaviors observed in animals prenatally exposed to this palatable food.

#### 4.1.4. Clinical evidence of a role of orexin/hypocretin in palatable food intake

—Clinical studies also connect OX with disturbances in palatable food intake, although the relationship may not be as direct as that observed in animals. The evidence demonstrates that individuals with narcolepsy who lack OX show significantly increased palatable food intake. They consume almost four times more calories than healthy matched controls when given *ad libitum* access to snacks (van Holst et al., 2016), and they score significantly higher than controls on a binge eating scale (Dimitrova et al., 2011). While these results run counter to those observed in animals which are examined primarily after short-term and reversible inhibition of the OX1R, it is possible that enhanced palatable food intake in narcoleptic patients reflects a behavioral pattern that overcompensates for the near total and chronic loss of OX function.

### 4.2. Role of orexin/hypocretin in alcohol drinking

The positive feedback relationship between OX and palatable food is also evident with alcohol. Since alcohol is a drug of abuse as well as a food that contains calories (7 kcal/g in ethanol, compared to 9 kcal/g in fat and 4 kcal/g in carbohydrates and protein), this substance has effects on the brain that resemble those produced by both drugs and food. As with palatable food, OX is found to promote the intake of alcohol in animals engaging in pharmacologically-relevant alcohol drinking that achieves blood alcohol levels sufficient to alter their behavior, and it does this primarily through extra-hypothalamic limbic areas where it also acts to enhance levels of dopamine in the nucleus accumbens. Similarly, OX levels are enhanced by alcohol intake, in a dose- and time-dependent manner, to promote further intake. Thus, OX and alcohol as with palatable food appear to interact in a positive feedback loop to contribute to substance overconsumption.

#### 4.2.1. Effects of orexin/hypocretin on aspects of alcohol drinking—

With models of access that induce pharmacologically-relevant drinking in the home cage, OX through both OX1R and OX2R has been found to promote alcohol intake, acting through a range of extra-hypothalamic limbic nuclei. These high-level drinking models involve intermittent

access to alcohol in the home cage and lead many subjects to binge on alcohol. The models include either the two-bottle choice procedure that provides 24-hour access to water plus 10-20% alcohol for 3 days per week (Carnicella, Ron, & Barak, 2014) or the "drinking in the dark" model that replaces the water bottle with a bottle of 20% alcohol for 2 to 4 hours (Thiele, Crabbe, & Boehm, 2014). With these intermittent access paradigms, peripheral administration of the OX1R antagonists SB-334867 and GSK1059865 are found to reduce alcohol intake and preference in both rats and mice (Lopez, Moorman, Aston-Jones, & Becker, 2016; Moorman & Aston-Jones, 2009; Olney, Navarro, & Thiele, 2015). Similarly, SB-334867 inhibits the lower-level drinking obtained from *ad libitum* home cage access (Anderson, Becker, Adams, Jesudason, & Rorick-Kehn, 2014). In contrast, systemic administration of the OX2R antagonist LSN424100 reduces the high-level alcohol intake from an intermittent access paradigm but has no effect on lower-level drinking (Anderson et al., 2014), indicating that the OX2R may be recruited along with OX1R only when animals drink alcohol at sufficiently high levels. Studies with local injections provide further evidence that both OX1R and OX2R affect high-level drinking, with OX1R acting in a wider range of brain regions. The OX1R antagonists reduce drinking in the dark when injected into the nucleus accumbens shell, central nucleus of the amygdala, or ventral tegmental area (Lei et al., 2016; Olney, Navarro, & Thiele, 2017) and intermittent access alcohol drinking when injected into the medial prefrontal cortex, but not the anterior insular cortex and substantia nigra (Lei et al., 2016; Srinivasan et al., 2012) or the paraventricular thalamus (Barson et al., 2015). This is in contrast to the OX2R antagonist TCS OX2 29, which reduces two-bottle choice alcohol drinking when injected into the paraventricular thalamus where OX-B increases it to a greater extent than an equimolar dose of OX-A (Barson et al., 2015; Barson et al., 2017), but which has no effect on drinking in the dark when injected into the nucleus accumbens shell, central nucleus of the amygdala, or ventral tegmental area (Lei et al., 2016; Olney et al., 2017). This evidence supports the idea that alcohol drinking is stimulated by OX1R in multiple limbic brain regions, while OX2R is most active in the paraventricular thalamus where it specifically promotes high-level drinking. In the hypothalamus, there is one study of low-level, *ad libitum* drinking in the home cage, which with OX-A injection shows that this peptide may also promote alcohol intake through this structure (Schneider, Rada, Darby, Leibowitz, & Hoebel, 2007). It is possible that OX within the hypothalamus promotes the intake of alcohol for its caloric content, in contrast to the extra-hypothalamic limbic regions where it promotes alcohol intake for its pharmacological effects.

Using a fixed ratio 3 schedule of reinforcement in an operant chamber, alcohol intake has also been found to be reduced by OX antagonists targeting either receptor. Thus, in various strains of rats, lever-pressing for alcohol is inhibited by peripheral injection of SB-334867 (Lawrence, Cowen, Yang, Chen, & Oldfield, 2006; Moorman, James, Kilroy, & Aston-Jones, 2017; Richards et al., 2008), the dual OX antagonist almorexant (Srinivasan et al., 2012), and the OX2R antagonist JNJ-10397049 (Shoblock et al., 2011). As with home-cage drinking, however, these receptor antagonists appear to exert their effects through different brain regions. This is suggested by the finding that operant self-administration of alcohol is reduced by injection of an OX1R antagonist into the shell of the nucleus accumbens (Lei et al., 2016) but by an OX2R antagonist in the core of the accumbens (Brown, Khoo, & Lawrence, 2013).

As with palatable food, OX increases not only the intake of alcohol but also an individual's willingness to work for alcohol, as measured by breakpoint in a progressive ratio task. Studies have implicated both receptor subtypes in this effect and suggest that this may be sex dependent. Specifically, the OX1R antagonist SB-334867 reduces breakpoint for alcohol in male but not female rats (Anderson et al., 2014; Jupp, Krivdic, Krstew, & Lawrence, 2011), whereas the OX2R antagonist LSN424100 reduces breakpoint in female rats (Anderson et al., 2014). Further studies are needed to determine if this reflects a sex difference in the recruitment of different brain regions during alcohol intake and the progressive ratio task.

Seeking for alcohol is also promoted by OX under several reinstatement paradigms and may be mediated by the OX1R. Cue-induced reinstatement of alcohol seeking is reduced or abolished by SB-334867 when injected systemically (Lawrence et al., 2006; Martin-Fardon & Weiss, 2014b; Moorman et al., 2017) or locally into the ventral tegmental area or medial prefrontal cortex (Brown et al., 2013). This contrasts with intracerebroventricular injection of the OX2R antagonist TCS OX2 29 which has no effect on this behavior (Brown et al., 2013). The possibility that OX participates in alcohol seeking under multiple relapse conditions is supported by the finding that systemic injection of the OX1R antagonist also inhibits yohimbine stress-induced relapse of alcohol seeking (Richards et al., 2008).

Despite the seemingly greater role of the OX1R in most aspects of alcohol drinking, the OX2R appears to be the primary receptor involved in reward from alcohol intake. In a conditioned place preference paradigm, where mice are conditioned to associate a distinct chamber with experimenter-administered alcohol, the OX2R antagonist JNJ-10397049 attenuates the acquisition, expression, and reinstatement of alcohol conditioned place preference (Shoblock et al., 2011). This is in contrast to the OX1R antagonist SB-334867 which, with the same dose of experimenter-administered alcohol, has no effect on the acquisition of alcohol conditioned place preference and blocks expression only of a weak place preference, but not a moderate or strong one (Voorhees & Cunningham, 2011). This suggests that reward following alcohol intake is primarily mediated by the OX2R.

**4.2.2. Effects of alcohol drinking on orexin/hypocretin—**In relation to consumption of alcohol, OX neuron activity, gene expression, and peptide levels vary in a time-dependent manner much as they do with the intake of palatable food. The activity of OX-expressing neurons is increased prior to voluntary alcohol drinking, consistent with the results of injection studies suggesting that this neuropeptide initiates and promotes alcohol intake. Specifically, levels of c-Fos are increased in OX neurons under conditions that induce relapse of alcohol seeking, including the presentation of an alcohol-paired cue (Dayas, McGranahan, Martin-Fardon, & Weiss, 2008), temporary inactivation of the nucleus accumbens (Millan, Furlong, & McNally, 2010), and placement into an alcohol-paired context (Moorman, James, Kilroy, & Aston-Jones, 2016). In response to alcohol intake, there is a change in OX that varies with dose and across time. While OX activity is inhibited by acute alcohol at a very high dose that promotes sleep (Sharma, Sahota, & Thakkar, 2014), similar to its inhibition after a physiological rise in glucose levels (Burdakov et al., 2005), gene expression and peptide levels of OX are significantly increased by alcohol administered at lower doses (Morganstern et al., 2010). Similarly, in response to voluntary intake, OX gene expression is stimulated during the first 30 minutes following the start of intake

(Barson et al., 2015; Lawrence et al., 2006; Sterling, Karatayev, Chang, Algava, & Leibowitz, 2015) but is subsequently unaffected or reduced along with OX peptide levels over the next several hours (Morganstern et al., 2010; Olney et al., 2015, 2017). This time-dependent shift in the effects of alcohol on OX, a stimulation followed by a suppression, may help to explain why individuals engage in alcohol binge sessions of a discrete duration (Carnicella et al., 2014), with drinking continuing until blood alcohol levels are sufficiently high to alter behavior and then ceasing until blood levels have significantly declined.

**4.2.3. Effects of prenatal exposure to alcohol on orexin/hypocretin—**With OX promoting pharmacologically-relevant alcohol drinking, it is notable that it is endogenously increased in animals prenatally exposed to alcohol. Prenatal exposure to low-to-moderate levels of alcohol leads to elevated alcohol drinking in adolescent and adult rodents and even zebrafish (Fabio, Macchione, Nizhnikov, & Pautassi, 2015; Nizhnikov, Popoola, & Cameron, 2016; Sterling, Chang, Karatayev, Chang, & Leibowitz, 2016). Notably, this exposure to alcohol also stimulates the proliferation of OX neurons, subsequently leading to their increase in gene expression and density (Chang, Karatayev, Liang, Barson, & Leibowitz, 2012; Sterling et al., 2016). This suggests that the greater number of neuropeptide neurons may contribute to elevated levels of alcohol intake.

**4.2.4. Clinical evidence of a role for orexin/hypocretin in alcohol drinking—**Individuals with OX-deficient narcolepsy show significant differences in their alcohol drinking. They have been found to be more likely to engage in frequent bouts of alcohol drinking compared to controls (Barateau et al., 2016), as they have with palatable food, possibly reflecting a compensatory mechanism for the chronic and significant loss of OX. However, this same study found these subjects to be less likely to engage in heavy alcohol drinking (Barateau et al., 2016), which is in line with a role for OX in promoting pharmacologically-relevant alcohol intake. Thus, in relation to alcohol, OX may be more involved in the excessive drinking that involves extra-hypothalamic limbic brain regions and feeds back to alter behavior, rather than the type of intake that involves the hypothalamus and is mainly for calories.

### 4.3. Role of orexin/hypocretin in nicotine intake

Literature exists that also connects OX with the use of nicotine in animals and cigarettes in humans. As with other rewarding substances, administration of OX promotes the intake of nicotine, with the specific brain regions involved yet to be determined, and endogenous OX levels are in turn enhanced by nicotine intake. This stimulatory effect of nicotine on OX has been observed at all time-points examined, suggesting more clearly than with palatable food and alcohol that OX interacts with nicotine in a positive feedback loop to enhance substance overconsumption.

**4.3.1. Effects of orexin/hypocretin on aspects of nicotine intake—**Evidence obtained primarily with systemic injection of OX receptor antagonists demonstrates that OX promotes not only the intake of nicotine but also the willingness to work for nicotine and the reinstatement of seeking for nicotine. Under a fixed ratio 5 schedule of reinforcement in an operant chamber, nicotine intake is reduced by the OX1R antagonist SB-334867 when

injected systemically (Hollander, Lu, Cameron, Kamenecka, & Kenny, 2008; LeSage, Perry, Kotz, Shelley, & Corrigall, 2010) or into the limbic insular cortex but not the adjacent somatosensory cortex (Hollander et al., 2008). These reports suggest a function for endogenous activity at the OX1R in the insular cortex in promoting the intake of nicotine, with further research needed to determine the involvement of other limbic brain regions and the OX2R in this behavior. Analysis of the willingness to work for nicotine indicates a role for OX1R but not OX2R, with lever-pressing for nicotine in a progressive ratio task found to be reduced by SB-334867 (Hollander et al., 2008) but not the OX2R antagonist 2-SORA (Uslaner et al., 2014). On the other hand, both receptors may participate in the seeking for nicotine under some reinstatement models, with intracerebroventricular injection of OX-A sufficient to reinstate previously extinguished nicotine seeking (Plaza-Zabala, Martin-Garcia, de Lecea, Maldonado, & Berrendero, 2010) and with SB-334867 and 2-SORA independently attenuating or blocking cue-induced reinstatement of nicotine seeking (Plaza-Zabala et al., 2013; Uslaner et al., 2014). These receptors do not appear to be involved in other nicotine reinstatement models, since the OX1R antagonist has no effect on footshock-induced reinstatement of nicotine seeking (Plaza-Zabala et al., 2010) and the OX2R antagonist has no effect on nicotine-induced reinstatement (Uslaner et al., 2014). These findings, consistent with those for palatable food and alcohol, support a role for OX in multiple aspects of nicotine intake behavior such as willingness to work and certain models of seeking, but the specific brain regions involved have yet to be determined.

As nicotine is a stimulant drug, its intake leads to both reward and anxiety, with these effects mediated in part by the OX1R (Hollander et al., 2008; Plaza-Zabala et al., 2010). Notably, the ability of nicotine to lower intracranial self-stimulation threshold is blocked by systemic administration of SB-334867 (Hollander et al., 2008). Similarly, the ability of nicotine to increase anxiety-like behavior in an elevated plus maze is also antagonized by SB-334867 (Plaza-Zabala et al., 2010). Thus, in addition to promoting the intake of nicotine, OX also plays a role in the emotional response to this intake.

**4.3.2. Effects of nicotine intake on orexin/hypocretin—**In relation to the intake of nicotine, OX neuron activity, gene expression, and peptide levels are elevated in an anatomically-distinct pattern, with the activity of OX-expressing neurons being higher before voluntary nicotine intake as well as after nicotine administration. Specifically, levels of c-Fos are increased in OX neurons following presentation of a cue predicting nicotine availability, in the perifornical and lateral hypothalamus but not the dorsomedial hypothalamus (Plaza-Zabala et al., 2013). This effect is also seen in the perifornical and dorsomedial hypothalamus after acute injection of nicotine at a relatively low dose (Plaza-Zabala et al., 2010) while in all populations of OX neurons after a higher dose of nicotine (Pasumarthi & Fadel, 2008; Pasumarthi, Reznikov, & Fadel, 2006). This difference in the areas recruited by a low versus high dose of nicotine may reflect a greater sensitivity of perifornical and dorsomedial hypothalamic neurons to acetylcholine. These effects on neuronal activity may also be specific to OX neurons, as they are not detected in adjacent neurons expressing melanin-concentrating hormone (Pasumarthi et al., 2006), and they are also evident *in vitro*, with acute nicotine application stimulating the firing of OX neurons (Huang, Xu, & van den Pol, 2011; W. L. Zhou, Gao, & Picciotto, 2015). Repeated nicotine

exposure *in vivo*, either through experimenter-administered injection or voluntary oral drinking, increases the gene expression of OX and peptide levels of both OX-A and OX-B in the hypothalamus (Kane et al., 2000; Tsuneki et al., 2016). While levels of OX-A are elevated only in the dorsomedial hypothalamus, the level of OX-B is increased in both the dorsomedial and paraventricular hypothalamus (Kane et al., 2000), suggesting that some of the major effects of nicotine are mediated by the OX2R. While not measured in extra-hypothalamic areas, it is likely that OX is similarly released into other limbic regions following nicotine intake, where it acts to further perpetuate this behavior.

**4.3.3. Effects of prenatal exposure to nicotine on orexin/hypocretin—**With OX both promoting and being activated by nicotine intake in adult animals, it is notable that this neuropeptide and nicotine consumption are similarly increased in animals exposed *in utero* to nicotine. Prenatal exposure to nicotine across a range of doses is shown to elevate nicotine intake in adolescent animals (Chang, Karatayev, & Leibowitz, 2013; Levin et al., 2006). Prenatal nicotine exposure at low doses (0.15 – 1.5 mg/kg/d) also increases OX gene expression and peptide levels (Chang et al., 2013; Morgan, Harrod, Lacy, Stanley, & Fadel, 2013), while the number of OX neurons and their gene expression are unaffected or reduced by nicotine at high levels (6 mg/kg/d) (Boychuk, Fuller, & Hayward, 2011). Further tests are needed to determine whether endogenous OX is indeed a critical driver of the elevated nicotine intake exhibited by offspring exposed early in life.

**4.3.4. Clinical evidence of a role for orexin/hypocretin in nicotine intake—**Changes in OX function have been associated with cigarette smoking in humans, although the direction of this association is not clear. For example, an allele for the OX2R has been linked to nicotine dependence in a Japanese population (Nishizawa et al., 2015). Also, individuals with OX-deficient narcolepsy who exhibit disturbances in their patterns of alcohol drinking are found to be more likely to smoke cigarettes than matched control subjects (Barateau et al., 2016). While the precise nature of the relationship remains to be resolved, these results provide some evidence linking OX to the use of nicotine.

### 4.4. Role of orexin/hypocretin in cocaine use

Research also connects OX with the use of cocaine, although the available studies suggest that this relationship is less direct than that observed with palatable food, alcohol, and nicotine. Possible reasons for the stronger and more direct connections between OX and these latter substances may be the fact that OX neurons are highly responsive to glucose that is elevated by palatable food intake (Burdakov et al., 2005), express both GABA receptors known to be strongly affected by alcohol intake (Backberg et al., 2003; Backberg et al., 2004), and also express nicotinic acetylcholine receptors strongly stimulated by nicotine intake (Garcia et al., 2015). In contrast, the relationship between OX and cocaine may be weaker or less direct due to the fact that OX neurons are found only rarely to express receptors for dopamine (Bubser et al., 2005), which is strongly elevated by cocaine intake. Thus, while able to promote various aspects of cocaine intake behavior, endogenous OX remains largely unaltered by the intake of cocaine, indicating that this drug may not directly affect OX neurons and alter their function.

**4.4.1. Effects of orexin/hypocretin on aspects of cocaine use—**Through investigations of the OX1R, endogenous OX has been found to promote the intake of cocaine, although only under specific conditions. Whereas intracerebroventricular injection of OX-A and systemic injection of the OX1R antagonist SB-334867 have no effect on operant responding for standard doses of cocaine under a fixed ratio 1 paradigm (Boutrel et al., 2005; Espana et al., 2010; Hutcheson et al., 2011; L. Zhou et al., 2012), there is other evidence suggesting that OX becomes involved when animals must work to attain their preferred blood levels of cocaine. Thus, SB-334867 significantly reduces fixed ratio 1 operant responding for very low doses of cocaine (Brodnik, Bernstein, Prince, & Espana, 2015) and also operant responding in a discrete trials paradigm involving highly restricted periods of access (Espana et al., 2010). Further, both SB-334867 and genetic knockout of the OX1R lead animals to consume less cocaine under a high work, fixed ratio 5 paradigm (Hollander, Pham, Fowler, & Kenny, 2012). The one study investigating the involvement of specific brain regions in mediating OX's actions found cocaine self-administration to be decreased by injection of SB-334867 into the central nucleus of the amygdala (Schmeichel, Herman, Roberto, & Koob, 2017). While this suggests that the OX1R in the amygdala may participate in promoting the intake of cocaine under conditions requiring work, a possible role for other limbic brain regions and the OX2R requires further investigation.

Not surprisingly, performance in a progressive ratio task that measures how much an individual is willing to work for cocaine is mediated by the OX1R. Specifically, the breakpoint for cocaine is reduced by peripheral injection of SB-334867 but not the OX2R antagonist 4PT (Borgland et al., 2009; Brodnik et al., 2015; Prince, Rau, Yorgason, & Espana, 2015). This effect of the OX1R appears to specifically involve the ventral tegmental area where injection of SB-334867 recapitulates the lowering of the breakpoint (Espana et al., 2010) and OX-A injection increases breakpoint (Espana, Melchior, Roberts, & Jones, 2011). With these injections into the ventral tegmental area also found, respectively, to inhibit or enhance the effects of cocaine on dopamine signaling (Espana et al., 2011; Espana et al., 2010), it is likely that dopamine mediates these changes in the amount of effort expended to obtain this drug.

As shown with the other rewarding substances, seeking for cocaine is also promoted by OX under a number of reinstatement paradigms and is mediated by the OX1R or OX2R depending on the brain region tested. Central injection of OX itself is sufficient to induce reinstatement of cocaine seeking, with this behavior mediated by the OX1R but not OX2R in the ventral tegmental area (B. Wang, You, & Wise, 2009) and by the OX2R but not the OX1R in the paraventricular thalamus (Matzeu, Kerr, Weiss, & Martin-Fardon, 2016). In contrast, OX antagonists are effective at inhibiting reinstatement of cocaine seeking after presentation of a cocaine-paired cue, return to the self-administration environment, or stress or yohimbine, but not after priming with cocaine itself which has minimal effects on OX levels (see Section 4.4.2). For example, cue-induced reinstatement of cocaine seeking is reduced by SB-334867 but not the OX2R antagonist 4PT when injected systemically (Hutcheson et al., 2011; Martin-Fardon & Weiss, 2014a; Smith, See, & Aston-Jones, 2009) or into the ventral tegmental area (James et al., 2011; Mahler, Smith, & Aston-Jones, 2013). Further, systemic injection of SB-334867 inhibits cocaine-seeking induced by context

(Smith, Tahsili-Fahadan, & Aston-Jones, 2010), footshock stress (Boutrel et al., 2005), or yohimbine (L. Zhou et al., 2012), with this latter reinstatement also reduced by SB-334867 injection into the central nucleus of the amygdala (Schmeichel et al., 2017). In contrast, neither SB-334867 nor 4PT affects the reinstatement of cocaine seeking induced by cocaine itself (Mahler et al., 2013; Smith et al., 2009; L. Zhou et al., 2012). These results demonstrate that the reinstatement of cocaine seeking is stimulated by OX in a variety of reinstatement paradigms, but not in drug-induced reinstatement.

Studies generally support the involvement of OX in the reward from cocaine intake. In a conditioned place preference paradigm in which animals are conditioned to associate a distinct chamber with experimenter-administered cocaine, the dual OX receptor antagonist almorexant is found to reduce the expression of this place preference (Steiner, Lecourt, & Jenck, 2013), similar to the effect produced by knockout of the OX gene (Shaw et al., 2016). Conversely, direct injection of OX-A into the ventral tegmental area is sufficient to reinstate an extinguished place preference (Tung et al., 2016). Further, cocaine-induced lowering of the intracranial self-stimulation threshold is also found to be reversed by systemic injection of SB-334867 in rats (Hollander et al., 2012), although this was not found to occur using a higher dose in mice (Riday et al., 2012). Overall, these results support a role for OX in the reward from cocaine intake.

**4.4.2. Effects of cocaine use on orexin/hypocretin—**In relation to the intake of cocaine, OX appears to be involved in behavior that occurs prior to its intake but this neuropeptide exhibits little change in response to its consumption. As with other rewarding substances, the activity of OX-expressing neurons is increased prior to seeking for cocaine, as shown by the finding that levels of c-Fos in OX neurons are increased by a cocaine-pared cue (Martin-Fardon, Cauvi, Kerr, & Weiss, 2016) or placement in a self-administration environment (Hamlin, Clemens, & McNally, 2008). In contrast to other substances, however, repeated injections of cocaine have little effect on OX gene expression in the hypothalamus (Y. Zhou et al., 2008), and acute injection of cocaine also fails to affect OX-A or OX-B peptide levels in the nucleus accumbens (Zhang, Mao, Liu, & Wang, 2007). These results provide further evidence that OX is involved in promoting cocaine intake but is not itself significantly affected by this behavior.

**4.4.3. Clinical evidence of a role for orexin/hypocretin in cocaine use—**Currently, there is only one study that has examined OX in relation to cocaine use in humans. Individuals with OX-deficient narcolepsy, who are found to show differences in their patterns of alcohol drinking and cigarette smoking, show no difference in their likelihood of using cocaine compared to matched control subjects (Barateau et al., 2016). This finding may be due to the low frequency of cocaine use among the sampled populations, pointing to the need for further translational research on this topic.

## 5. Conclusions

While OX was originally identified for its role in food intake and energy homeostasis, it has since become clear that OX participates in a range of behaviors, which are both homeostatically- and non-homeostatically-regulated. Many of the behaviors promoted by

OX can be classified as adaptive, including food intake, sleep-wake regulation, mating, and maternal behavior. These behaviors are largely controlled by the release of OX in the hypothalamus, with food intake involving the same regions of the hypothalamus as OX transcription (perifornical, dorsomedial, and lateral areas), waking additionally involving areas like the locus coeruleus, and mating and maternal behavior involving the medial preoptic area of the hypothalamus. The enactment of these adaptive behaviors results in feedback signals such as glucose that negatively regulate OX activity, signaling the completion of the behavioral sequence. We propose that OX in promoting these adaptive behaviors under physiological conditions has an important role in maintaining arousal to promote survival, both invigorating and motivating behavior necessary for the propagation of an individual's genes. Further studies uncover another property of OX, its ability to promote overconsumption of rewarding substances of abuse, such as palatable food, alcohol, nicotine, and cocaine, with this peptide both stimulating and being stimulated by the intake of these substances. This function of OX occurs through a different set of limbic nuclei outside of the hypothalamus, most notably the nucleus accumbens, ventral tegmental area, and paraventricular nucleus of the thalamus, in addition to the amygdala, prefrontal cortex, and insular cortex. Through these extra-hypothalamic limbic brain regions, OX promotes the intake, willingness to work, seeking, and reward derived from the intake of rewarding substances. These effects are mediated by activity at the OX1R in most limbic regions, except for the paraventricular thalamus where they are mediated by OX2R. Moreover, in response to the intake or early life exposure to these rewarding substances, OX gene expression and peptide levels are generally further elevated, forming a vicious cycle involving positive feedback rather than the negative feedback characteristic of homeostatic cycles. Together, these results suggest that the OX system in a broad range of extra-hypothalamic limbic brain areas can be coopted by rewarding substances, such as palatable food, alcohol, nicotine, and cocaine, to drive intake non-homeostatically and ultimately lead to addiction.

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