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REVIEW

Orexin/hypocretin role in reward: implications for opioid and other addictions

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Addiction is a devastating disorder that affects 15.3 million people worldwide. While prevalent, few effective treatments exist. Orexin receptors have been proposed as a potential target for anti-craving medications. Orexins, also known as hypocretins, are neuropeptides produced in neurons of the lateral and dorsomedial hypothalamus and perifornical area, which project widely throughout the brain. The absence of orexins in rodents and humans leads to narcolepsy. However, orexins also have an established role in reward seeking. This review will discuss some of the original studies describing the roles of the orexins in reward seeking as well as specific works that were presented at the 2013 International Narcotics Research Conference. Orexin signalling can promote drug-induced plasticity of glutamatergic synapses onto dopamine neurons of the ventral tegmental area (VTA), a brain region implicated in motivated behaviour. Additional evidence suggests that orexin signalling can also promote drug seeking by initiating an endocannabinoid-mediated synaptic depression of GABAergic inputs to the VTA, and thereby disinhibiting dopaminergic neurons. Orexin neurons co-express the inhibitory opioid peptide dynorphin. It has been proposed that orexin in the VTA may not mediate reward *per se*, but rather occludes the 'anti-reward' effects of dynorphin. Finally, orexin signalling in the prefrontal cortex and the central amygdala is implicated in reinstatement of reward seeking. This review will highlight recent work describing the role of orexin signalling in cellular processes underlying addiction-related behaviours and propose novel hypotheses for the mechanisms by which orexin signalling may impart drug seeking.

LINKED ARTICLES

This article is part of a themed section on Opioids: New Pathways to Functional Selectivity. To view the other articles in this section visit http://dx.doi.org/10.1111/bph.2015.172.issue-2

Abbreviations

BOLD, blood oxygen level-dependent; CeA, central nucleus of the amygdala; CPP, conditioned place preference; CRF, corticotropin-releasing factor; DAGL, DAG lipase; DMH, dorsomedial nucleus of the hypothalamus; LH, lateral hypothalamus; mPFC, medial prefrontal cortex; NAc, nucleus accumbens; PFA, perifornical area; VTA, ventral tegmental area

Introduction

Neurons containing orexin, also known as hypocretin, are firmly established as regulators of reward seeking. Orexin-A and -B (hypocretin-1 and -2) are peptides produced in neurons residing in the lateral and dorsomedial hypothalamus and perifornical area (de Lecea *et al.*, 1998; Sakurai *et al.*, 1998) that have widespread projections throughout the brain (Peyron *et al.*, 1998). Orexin containing neurons project from the lateral hypothalamus (LH) to many areas of the mesolimbic 'reward pathway' including the ventral tegmental area (VTA) and the nucleus accumbens (NAc) (Di Chiara and Imperato, 1985; Koob and Bloom, 1988; Wise and Rompre, 1989) and these neurons are primarily implicated in reward-seeking behaviour. Within the VTA, 20% of neuronal inputs originating in the LH show immunolabelling for orexin (Balcita-Pedicino and Sesack, 2007). Moreover, orexin fibres co-distribute with dopamine fibres in both the medial prefrontal cortex (mPFC) and the medial shell of the NAc (Fadel and Deutch, 2002) suggesting that orexin and dopamine may interact at several levels in the reward system. Orexin innervation is present throughout the entirety of the VTA. Approximately 15% of orexin axons make appositional contacts in the VTA with 5% showing identifiable synaptic specializations onto both dopaminergic and GABAergic neurons (Balcita-Pedicino and Sesack, 2007). Because a function of many neuropeptides, including orexin, is the modulation of fast amino acid neurotransmitter signalling, and neuropeptide release is not limited to synaptic specializations, the low synaptic incidence in the VTA is likely to be not relevant to the ability of orexin to modulate the activity of VTA dopaminergic and GABAergic neurons. This is supported by the observation that orexin axons contain many dense core vesicles within the VTA (Balcita-Pedicino and Sesack, 2007), which are capable of exocytosis at extra-synaptic sites. From these sites, orexin can diffuse small distances to modulate nearby synapses. Orexin neurons co-express glutamate and the vesicular glutamate transporters VGlut1 and VGlut2 (Rosin *et al.*, 2003; see Alexander *et al.*, 2013a). Thus, the orexin-containing neurons that do synapse in the VTA likely represent a minor source of glutamate (Figure 1). Finally, orexin neurons also co-express the opioid dynorphin (Chou *et al.*, 2001), and therefore may co-release dynorphin in the VTA or at other projection targets. This review aims to discuss (i) the involvement of hypothalamic orexin neurons in drug taking, seeking and withdrawal; (ii) the role of orexin receptor signalling in the VTA and ascending projection targets on drug seeking and reinstatement of drug seeking; and (iii) the possible role of co-released orexin and dynorphin on drug reward. Furthermore, specific work that was presented at the 2013 International Narcotics Research Conference (INRC) will be highlighted.

Differential effects of OX_1 and OX_2 receptors on drug seeking

Once released, orexins elicit their effects via two GPCRs: the orexin-1 (OX_1) receptor and orexin-2 (OX_2) receptor (Sakurai *et al.*, 1998; receptor nomenclature follows Alexander *et al.*, 2013a). Both OX_1 and OX_2 receptors are somewhat promiscuous in their G-protein signalling with evidence linking both receptor subtypes to G_q, G_s and $G_{i/o}$ interactions (Kukkonen and Leonard, 2014). Although the G_q pathway appears to have important functional roles, a lack of direct methods to measure G-protein activation has inhibited a definitive assessment of orexin receptor G-protein coupling (see Kukkonen and Leonard, 2014). In line with the extensive orexinergic projection fields throughout the neuraxis, OX_1 and OX_2 receptors are widely distributed within the brain (Trivedi *et al.*, 1998). For example, OX_1 receptors are more highly expressed in cortical regions, the bed nucleus of the stria terminalis and the locus coeruleus, whereas OX_2 receptor density is enriched over OX_1 receptors in the NAc and specific thalamic/hypothalamic regions. The VTA contains relatively similar expression of both receptors (Sakurai *et al.*, 1998; Trivedi *et al.*, 1998).

Recent evidence suggests a dichotomous role of OX_1 and OX_2 receptors in the brain. OX_1 receptors are important for the neurobiological effects that drive drug seeking for morphine (Harris *et al.*, 2005; 2007), cocaine (Harris *et al.*, 2005; Borgland *et al.*, 2006), nicotine (Pasumarthi *et al.*, 2006; Hollander *et al.*, 2008; Plaza-Zabala *et al.*, 2010; 2012) and alcohol (Lawrence *et al.*, 2006; Dayas *et al.*, 2008; Richards *et al.*, 2008; Moorman and Aston-Jones, 2009; Jupp *et al.*, 2011b; Kim *et al.*, 2012; Srinivasan *et al.*, 2012). In contrast, OX_2 receptors have been implicated more strongly in sleep/wake cycle regulation and arousal (Willie *et al.*, 2003). While some studies suggest that OX_2 receptors play a lesser role in drug seeking (Smith *et al.*, 2009; Shoblock *et al.*, 2011), the role of OX_2 receptors in ethanol reinforcement and ethanol-seeking behaviour is less clear. To determine the role of OX_2 receptors in ethanol-taking and ethanol-seeking behaviour, rats were trained to self-administer ethanol (10% w/v) or sucrose (0.7–1% w/v) in the presence of reward-associated cues. i.c.v. administration of the selective OX_2 receptor antagonist TCS-OX2-29 reduced self-administration of ethanol, but not sucrose, and had no effect on cue-induced reinstatement of ethanol seeking (Brown *et al.*, 2013). Furthermore, intra-NAc core, but not shell, infusions of TCS-OX2-29 decreased responding for ethanol (Brown *et al.*, 2013). Thus, OX_2 in addition to OX_1 receptors may represent a potential therapeutic target for the treatment of alcohol use disorders. However, unlike OX_1 receptors, no effect of OX_2 receptor antagonism was observed on cue-induced reinstatement of ethanol seeking, suggesting a more prominent role for OX_2 receptors in ethanol self-administration compared with cue-conditioned ethanol seeking. Importantly, however, these data are restricted to cue-driven ethanol seeking; it is quite possible that OX_2 receptors may be implicated in other forms of reward seeking, such as stress-mediated or drug-primed reward seeking. Future studies are therefore required to further elucidate the varied roles of OX_1 and OX_2 receptors in cue, stress or drug-primed reward seeking, including identification of the anatomic loci and mechanisms underlying these effects. Furthermore, while morphine seeking and withdrawal are dependent on OX_1 receptor signalling (Georgescu *et al.*, 2003; Harris *et al.*, 2005), no studies have addressed whether OX_2 receptors are required for morphine seeking, reinstatement or withdrawal.

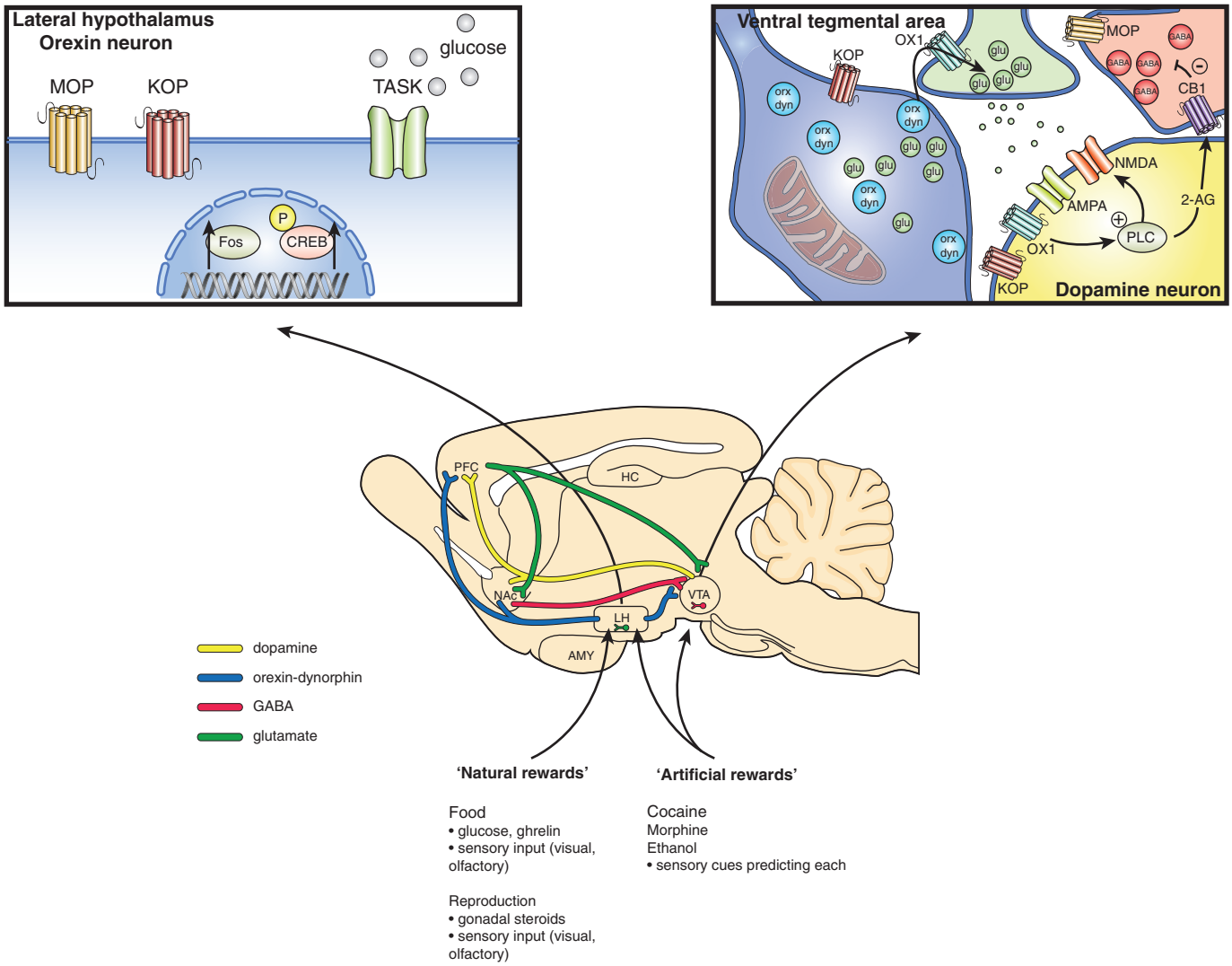

Figure 1

Simplified diagram of pathways involved in orexin signalling. Neurohumoural information about natural rewards is integrated by orexin neurons of the LH. Drugs of abuse may act both locally in the LH and at orexin terminals in VTA. Exposure to rewards or reward cues increases Fos expression and phospho-CREB in orexin neurons (inset, upper left). In the VTA (inset, upper right) orexin can affect glutamate release onto dopaminergic DA neurons as well as stimulating them directly via postsynaptic OX_1 receptors. Within DA neurons, OX_1 activation can recruit synthetic enzymes (PLC) for endocannabinoids such as 2-AG, which can inhibit GABA release from interneurons in a retrograde fashion. Co-released dynorphin may also modulate the excitatory effects of its co-transmitter orexin. MOP, KOP; μ-opioid, κ–opioid receptors: TASK, K_{2P} potassium channels.

An fMRI study in rodents demonstrated that selective antagonists to either OX_1 or OX_2 receptors inhibited blood oxygen level-dependent (BOLD) responses to amphetamine. However, the selective OX_1 receptor antagonist modulated functional responses in the striatum, whereas the OX_2 receptor antagonist attenuated the amphetamine-induced response predominantly in the cortex (Gozzi *et al.*, 2011). In a subsequent experiment, the same doses of the OX_2 receptor antagonist were strongly sedating, while the OX_1 receptor antagonist significantly reduced a cocaine-induced conditioned place preference (CPP) (Gozzi *et al.*, 2011). Thus, the functional differences observed between OX_1 and OX_2 receptors on different aspects of drug seeking or arousal may be due to differential regional expression of these receptors in the brain.

Hypothalamic orexin neurons are activated by drugs, context, stress and withdrawal

The LH has been studied for its role in motivated behaviour for more than half a century (Hess, 1954). Early investigators found that non-contingent electrical stimulation of this tissue could evoke a wide variety of behavioural responses

from simple increases in ambulation to more complex, goal-directed activities like feeding, drinking or copulation (Valenstein *et al.*, 1970; Valenstein, 1973; Hoebel, 1976). These studies reached their apotheosis with the discovery that rodents (and indeed humans) would vigorously perform arbitrary operant responses for electrical stimulation to the LH (Olds and Milner, 1954; Heath, 1963). Since this time, the LH has been recognized as a critical node in a complex circuit that regulates reinforcement and reward (Kauer and Malenka, 2007). The LH appears to control responding not only for natural rewards like food or receptive mates, but also pharmacological rewards like drugs of abuse.

There is not only a close resemblance between the anatomical distribution of the orexin neuronal population and sites in the LH known to support rewarding self-stimulation (Valenstein *et al.*, 1970; Peyron *et al.*, 1998; Hollander *et al.*, 2008; 2012), but also for the functional effects of orexin peptides when compared with LH stimulation. Indeed, central administration of orexin peptides evokes many of the same behaviours observed decades before with electrical stimulation of the LH (see Table 1). These observations prompted research on the role of orexin peptides in reward and motivated behaviour. This line of inquiry has been fruitful. A body of literature has emerged supporting the view that the orexin system fulfils a central role in integrating signals from the periphery conveying information about macronutrient balance (Burdakov *et al.*, 2006; Karnani *et al.*, 2011), and hormonal competence for reproductive behaviour (Muschamp *et al.*, 2007; Di Sebastiano *et al.*, 2011), with circadian information (Estabrooke *et al.*, 2001; Mileykovskiy *et al.*, 2005) in order to coordinate arousal levels appropriate for the acquisition of food or receptive mates (Adamantidis and de Lecea, 2008). These goal-directed behaviours are engaged, in part, via robust projections of the orexin neurons to reward-responsive dopamine neurons in the VTA (Fadel and Deutch, 2002; Balcita-Pedicino and Sesack, 2007). This natural reward circuitry can be co-opted by repeated exposure to drugs of abuse, resulting in increased drug-seeking behaviour and the emergence of a set of neural adaptations that are putative markers of drug dependence.

Drug-induced Fos activation in orexin neurons

The anatomical inputs to, and outputs from, orexin neurons make them an ideal candidate for mediating certain aspects of reward and motivation. Therefore, many studies have examined the role of orexin neuron activity in drug-seeking behaviours. Harris *et al.* (2005) first reported that orexin neurons in the LH, but not the dorsomedial nucleus of the hypothalamus (DMH) or the perifornical area (PFA), were activated by cues associated with both drug (cocaine and morphine) and food rewards. In a CPP task, whereby animals learn to associate distinct contextual cues with the subjective effects of experimenter-administered drugs, preference for the drug-paired environment positively correlates with the level of Fos protein, a surrogate marker of neural activation, in LH orexin neurons whereas animals showing no preference have Fos levels similar to controls (Harris *et al.*, 2005). Interestingly, this effect seems to be distinct from novel object preference, which does not activate orexin neurons in the LH (Harris *et al.*, 2005). Moreover, stimulation of the LH, or microinfusion of orexin-A into the VTA, can reinstate an extinguished CPP; with both effects being inhibited by the selective OX_1 receptor antagonist, SB 334867 (Harris *et al.*, 2005). Conversely, foot shock, a stressful stimulus that can also reinstate drug seeking, does not activate LH orexin neurons but rather activates those in the DMH and PFA (Harris *et al.*, 2005). Together, these results led to the hypothesis that discrete populations of orexin neurons display a functional dichotomy in responses to pharmacological or environmental stimuli, and in turn, to organizing behaviour. It was suggested that orexin neurons situated in the DMH and PFA might contribute to behaviours linked with arousal and foot shock stress (Harris and Aston-Jones, 2006; Sharf *et al.*, 2010) whereas those located in the LH might have a preferential role in reward seeking (Harris and Aston-Jones, 2006). If so, this dichotomy might arise from preferential targeting

Table 1

Electrical stimulation of lateral hypothalamus and central infusion of orexins (hypocretins) both activate a diverse array of behaviours in rats

Behaviour	LH stimulation citation	Central orexin (hypocretin) citation	Injection site	Dose
Feeding	Hoebel, 1969; Hoebel *et al.*, 1962; Margules *et al.*, 1962	Dube *et al.*, 1999 Haynes *et al.*, 1999 Sakurai *et al.*,1998	LH, PVN, DMH i.c.v. i.c.v.	1 nmol Hcrt-1/Orx A 23.4 nmol Hcrt-1/Orx A 3, 30 nmol Hcrt-1/Orx A 3, 30 nmol Hcrt-2/Orx B
Drinking	Mogenson and Stevenson, 1967	Kunii *et al.*,1999	i.c.v.	10 nmol Hcrt-1/Orx A 10 nmol Hcrt-2/Orx B
Copulation	Caggiula *et al.*, 1966; Herberg, 1963; Vaughan *et al.*, 1962	Gulia *et al.*, 2003	mPOA	0.3 nmol Hcrt-1/Orx A
Locomotion	Rolls *et al.*, 1972	Kotz *et al.*, 2002	LH	1 nmol Hcrt-1/Orx A
Gnawing of non-food objects	Valenstein, 1973; Valenstein *et al.*, 1968	España *et al.*, 2002	i.c.v.	3 nmol Hcrt-1/Orx A

mPOA, medial preoptic area; PVN, paraventricular nucleus of hypothalamus.

of orexin neurons within each subregion; such that orexin neurons in the DMH and PFA may mediate arousal by projecting to arousal-related regions such as the locus coeruleus; and LH orexin neurons may influence reward through projections to the brain reward circuitry including, but not limited to, the VTA (Harris and Aston-Jones, 2006). However, this idea has been contradicted by a recent report that orexin neurons projecting both to arousal and reward-related areas, the locus coeruleus and VTA, respectively, are not segregated to the DMH/PFA and LH but rather are spread throughout the entire orexin field (González *et al.*, 2012). In fact, VTA-projecting orexin neurons outnumber locus coeruleus-projecting neurons in the medial portions of the orexin field (González *et al.*, 2012). In addition, amphetamine-sensitized rats show c-Fos+orexin double labelling in all three subregions of the orexin field (McPherson *et al.*, 2007) and restraint stress was found to reinstate extinguished cocaine seeking via activation of LH, but not PFA orexin neurons (Tung and Chiou, 2012). Therefore, an alternative hypothesis has been proposed whereby differential activation of orexin neurons may be a result of distinct upstream activation of afferent projections to orexin neurons. In line with this hypothesis is the finding that orexin neurons in the medial regions are preferentially innervated by hypothalamic neurons while those in the LH receive preferential afferent input from the brain stem and reward-related regions (Yoshida *et al.*, 2006).

In this light, Sartor and Aston-Jones (2012) characterized which afferents to LH orexin neurons are activated by reward-related stimuli, and if these inputs could drive activity in orexin neurons to promote drug place preference. They focused on the lateral septum, a brain region that sends afferents to the LH (Yoshida *et al.*, 2006), and is activated by drugs and environmental information associated with reward (Shoji *et al.*, 1997; 1998). Here, they demonstrated that neurons projecting from the rostral, but not the caudal, lateral septum to the LH, but not to the DMH or PFA, were Fos activated following cocaine CPP and that the percentage of Fos activation correlated with preference scores (Sartor and Aston-Jones, 2012). Importantly, this circuit may be a small part of a more complex circuit in which the lateral septum integrates contextual information with motivational processes (Sartor and Aston-Jones, 2012). Therefore, it will be interesting to see if these data hold true for other rewards or drugs of other pharmacological classes, like morphine.

Withdrawal-induced Fos activation in hypothalamic orexin neurons

Morphine withdrawal induced by naloxone administration increases Fos activation of LH orexin neurons in mice (Georgescu *et al.*, 2003) and rats (Laorden *et al.*, 2012). In contrast to Fos expression induced by morphine CPP (Harris *et al.*, 2005), Fos expression induced by morphine withdrawal was localized to DMH and PFA, but was absent in LH orexin neurons of mice (Georgescu *et al.*, 2003). However, this was not the case in rats where withdrawal also induced Fos expression in the LH (Laorden *et al.*, 2012). Interestingly, morphine withdrawal increased both orexin and μ-opioid receptor mRNA selectively in the LH (Zhou *et al.*, 2006). Increased Fos induction by morphine withdrawal was attenuated in orexin peptide knockout mice (Sharf *et al.*, 2008) or in wild-type mice (Georgescu *et al.*, 2003) or rats (Laorden *et al.*, 2012) treated with an OX_1 receptor antagonist. Finally, systemic SB 334867 inhibited morphine withdrawal-induced Fos expression in the hypothalamus, NAc shell, bed nucleus of the stria terminalis and central amygdala, but not the VTA or the locus coeruleus (Sharf *et al.*, 2008; Laorden *et al.*, 2012). Surprisingly, direct infusion of SB 334867 into the locus coeruleus attenuated somatic signs of morphine withdrawal in rats (Azizi *et al.*, 2010). These results suggest that, in addition to conditioned drug seeking, hypothalamic orexin neurons are also activated during morphine withdrawal.

Drug-induced plasticity at orexin neurons

In addition to drug-induced Fos activation of orexin neurons, drugs have been reported to alter synaptic transmission within the LH, including that onto orexin neurons. Acute application of Met-enkephalin or morphine can depress the firing rate and the frequency of miniature EPSCs (mEPSCs) onto orexin neurons (Li and van den Pol, 2008). Furthermore, 1 h pre-exposure to morphine reduced morphine-induced inhibition of firing 30 min later (Li and van den Pol, 2008). So far, it has not been determined how *in vivo* morphine exposure can modify synaptic transmission or plasticity of orexin neurons.

Drug-induced synaptic plasticity in reward-associated areas including the VTA and the NAc is essential to both the development and the expression of drug-related behaviours (reviewed in Lüscher and Malenka, 2011). LH hypothalamic neurons also appear to undergo drug-induced plasticity. Recent reports from Rao *et al.* (2013) and Yeoh *et al.* (2012) described cocaine-induced plasticity of glutamatergic inputs to orexin neurons. In mice that received repeated cocaine in a CPP paradigm, there was a long-lasting increase in the AMPA/NMDA receptor ratio, a measure of synaptic strength, as well as the amplitude of AMPA receptor mEPSCs, suggesting an increase in postsynaptic AMPA receptor expression (Rao *et al.*, 2013; receptor nomenclature follows Alexander *et al.*, 2013b). In contrast, Yeoh *et al.* (2012) observed enhanced excitatory inputs to PFA/LH neurons including orexin neurons, but no postsynaptic changes, after 7 days of cocaine self-administration in rats. The difference in these responses may be due to the duration or method of cocaine delivery (3 days vs. 7 days and experimenter-administered vs. self-administered cocaine). This plasticity at synapses onto orexin neurons was not dependent on OX_1 receptor signalling as cocaine-induced strengthening of glutamatergic synapses onto orexin neurons was not blocked by systemic SB 334867 (Rao *et al.*, 2013). This finding differentiates drug-induced plasticity in the LH from that at dopamine neurons in the VTA, which is dependent on OX_1 receptor signalling (Borgland *et al.*, 2006), as discussed below.

Effects of orexin receptor activation in the VTA

Although there is clear evidence of drug-induced modulation of orexin neurons, it is the downstream release of orexins in target projection regions that likely represents a critical step in the initiation and maintenance of drug-seeking behaviour. Highlighting this is the finding that cocaine CPP is inhibited

by SB 334867 despite the persistence of cocaine-induced plasticity of orexin neurons (Rao *et al.*, 2013). Therefore, plasticity at the level of orexin neurons is likely to represent a mechanism to increase the excitability of orexin neurons in order to enhance the probability of downstream release. At these downstream sites, orexin can modulate synaptic transmission and influence motivated behaviour.

Acute effects of orexin in the VTA

In whole cell patch-clamp electrophysiology experiments using midbrain slices, direct application of orexin-A or orexin-B increased the firing rate of VTA dopamine neurons (Korotkova *et al.*, 2003). Furthermore, orexin-A or -B concentration dependently increased NMDA receptor EPSCs via a PKC/PLC-dependent signalling pathway (Figure 1; Borgland *et al.*, 2006; 2008). Although acute application of orexin-A had no effect on AMPA receptor EPSCs when measured 15 min later, both the amplitude of AMPA receptor mEPSCs and the AMPA/NMDA receptor ratio were increased 3–4 h later, suggesting that acute potentiation of NMDA receptor currents led to a late phase potentiation of AMPA receptors. Accordingly, an NMDA receptor antagonist blocked the increase in AMPA receptor mEPSC amplitude (Borgland *et al.*, 2006). Thus, orexin-A potentiation of NMDA receptor EPSCs may serve to facilitate the induction of synaptic plasticity at VTA dopaminergic neurons.

Chiou *et al.* (2013) proposed another cellular mechanism involving endocannabinoids to explain how orexin increases VTA dopaminergic activity. Endocannabinoids, especially 2-arachidonoylglycerol (2-AG), are synthesized and then released locally in an activity-dependent and receptor-regulated manner (Di Marzo *et al.*, 1994). Endocannabinoids can be generated when certain GPCRs coupled to $G_{q/11}$, including OX_1 receptors, are activated (Kano *et al.*, 2009; Ho *et al.*, 2011), resulting in retrograde inhibition of neurotransmitter release via activation of presynaptic cannabinoid CB_1 receptors (Kano *et al.*, 2009). In a slice electrophysiology study, Chiou *et al.* (2013) demonstrated that activation of OX_1 receptors on VTA dopaminergic neurons could initiate the synthesis of 2-AG by postsynaptic DAG lipase (DAGL) and subsequent retrograde signalling onto CB_1 receptor-expressing GABAergic terminals (Matyas *et al.*, 2008). Orexin-A depressed inhibitory postsynaptic currents (IPSCs) via a presynaptic mechanism. This effect was (i) inhibited by OX_1 and CB_1, but not OX_2, receptor antagonists; (ii) prevented by inhibitors of G-protein signalling, PLC or DAGL; and (iii) potentiated and prolonged by inhibiting the major 2-AG degrading enzyme, monoglycerol lipase (Ludanyi *et al.*, 2011). These results suggest that in VTA dopaminergic neurons, orexin-A initiates a $G_{q/11}$PCR-coupled PLC-DAGL enzymic pathway to generate 2-AG, which then travels retrogradely across the synapse to inhibit GABA release through presynaptic CB_1 receptors (unpublished observations presented at the 2013 INRC meeting; Chiou *et al.*, 2013; Figure 1). This is likely to result in disinhibition of VTA dopamine neurons.

Acute application of orexin-A or -B increases the firing rate of GABAergic neurons (Korotkova *et al.*, 2003), which presumably decreases VTA dopaminergic activity. Indeed, in a preliminary study, Tung and Chiou (2012) found that orexin-A depressed IPSCs in VTA dopaminergic neurons in a bell-shaped concentration–response curve. In the presence of a CB_1 receptor antagonist, higher concentrations of orexin-A potentiated IPSCs whereas lower concentrations depressed IPSCs. This suggests that higher concentrations of orexin-A are needed to directly activate GABAergic neurons, as compared with the concentrations that initiate 2-AG-mediated retrograde disinhibition. Interestingly, orexin-A and -B were equally effective at increasing the firing rate of GABAergic neurons in the VTA (Korotkova *et al.*, 2003). Given that OX_1 receptors display higher affinity for orexin-A than orexin-B, whereas OX_2 receptors have equal affinity for orexin-A and -B (Sakurai *et al.*, 1998), it remains to be elucidated if the direct neuronal excitatory effect of orexin is mainly mediated by OX_2 receptors while OX_1 receptors may mediate the generation of 2-AG and its subsequent signalling cascade.

Drug-induced modulation of synaptic transmission in the VTA is thought to promote a switch from tonic to burst firing, a phenomenon that increases the efficiency of dopamine release in target regions (Overton and Clark, 1991; Suaud-Chagny *et al.*, 1992; Tong *et al.*, 1996). Indeed, orexin-A or orexin-B application to VTA midbrain slices increased dopamine neuron firing and in some cases was capable of inducing burst firing activity (Korotkova *et al.*, 2003). However, dopaminergic neurons were not uniformly excited by orexin application as a substantial number of dopaminergic neurons were unaffected by orexin application (Korotkova *et al.*, 2003). With the emerging notion of heterogeneity of dopaminergic neurons in the VTA both in terms of cellular properties and circuit level connections (Lammel *et al.*, 2008; 2011; 2012; Margolis *et al.*, 2008), such a varied response to orexin application within the VTA could suggest that orexin functions within specific circuits in the VTA to modulate different aspects of motivated behaviour. Accordingly, orexin-A preferentially activates VTA dopaminergic neurons that project to the mPFC and the NAc shell (Vittoz *et al.*, 2008). Regardless of differential activation of dopamine neurons in the VTA by orexin, intra-VTA application of orexin-A is sufficient to increase dopamine release in the mPFC (Vittoz and Berridge, 2006; Vittoz *et al.*, 2008) and the NAc (Narita *et al.*, 2006; 2007; España *et al.*, 2011).

Effect of orexin signalling on drug-induced plasticity in the VTA

The ability of orexin to induce synaptic plasticity in the VTA may represent a mechanism by which environmental drug-related stimuli, known to activate orexin neurons (Harris *et al.*, 2005; 2007), modulate excitatory transmission in the VTA. Morphine, cocaine and other drugs of abuse strengthen AMPA-mediated synaptic transmission onto dopamine neurons when tested 24 h after drug exposure (Ungless *et al.*, 2001; Saal *et al.*, 2003; Borgland *et al.*, 2004), an effect that is dependent on NMDA receptor activation (Ungless *et al.*, 2001; Argilli *et al.*, 2008). Interestingly, systemic administration of the OX_1 antagonist SB 334867 blocks cocaine-induced synaptic plasticity in the VTA (Borgland *et al.*, 2006). Preliminary evidence suggests that SB 334867 administered systemically or within the VTA also blocks morphine-induced plasticity at VTA dopamine neuronal synapses (unpublished observations presented at the 2013 INRC meeting). These results are interesting as acute application of morphine depresses firing and glutamatergic activity onto orexin

neurons, implicating reduced orexin release (Li and van den Pol, 2008). However, further studies are required to determine how *in vivo* exposure to morphine subsequently modifies activity of orexin neurons. Nevertheless, it appears that drug-induced plasticity of VTA dopaminergic neurons requires orexin signalling, regardless of the mechanism of action of the drug. Interestingly, both systemic and intra-VTA SB 334867 administration attenuates cocaine-induced increases in dopamine concentrations in the NAc core (España *et al.*, 2010), while intra-VTA orexin-A infusions increase dopamine concentrations and the efficacy of cocaine-mediated dopamine reuptake inhibition (España *et al.*, 2011). Taken together, blockade of orexin-A signalling in the VTA can inhibit cocaine-mediated strengthening of synapses and output of VTA dopaminergic neurons (España *et al.*, 2010). Current studies are underway to determine if systemic or intra-VTA SB 334867 can prevent morphine-induced increases in terminal dopamine release.

Drug-induced plasticity in the VTA is thought to underlie some of the core behavioural features of addiction. For example, locomotor sensitization, which manifests as a progressive and persistent increase in locomotor activity with repeated administration of a drug (Robinson and Berridge, 1993), is dependent on NMDA receptor activation in the VTA (Kalivas and Alesdatter, 1993) and results in increased dopaminergic transmission. Consistent with the effects of orexin on NMDA receptors in the VTA, the development of morphine-, cocaine- and amphetamine-induced behavioural sensitization is also inhibited by the systemic administration of an OX_1 receptor antagonist (Borgland *et al.*, 2006; Narita *et al.*, 2006; Quarta *et al.*, 2009).

While acute application of orexin has prominent effects on synaptic transmission in the VTA, long-term drug exposure further alters these effects. Rats that self-administered cocaine or high-fat food had potentiated orexin-induced increases in NMDA receptor currents compared with those that received regular food (Borgland *et al.*, 2009). This was observed along with an increase in AMPA receptor mEPSC frequency in these animals, suggesting that prolonged exposure to a positive reinforcer induces a presynaptic increase in glutamate release that is absent in naïve animals or those with a history of self-administering a less-salient reinforcer (Borgland *et al.*, 2009). Further studies are required to assess if opioid self-administration, another salient reinforcer, also promotes an orexin-mediated increase in excitatory synaptic transmission at dopaminergic synapses.

Effects of orexin receptor activation in the VTA on drug seeking and reinstatement

Several lines of evidence suggest that endogenous orexins acting via OX_1 receptors in the VTA play an important role in the reinstatement of extinguished reward seeking. First, activation of LH orexin neurons, which send substantial projections to the VTA (Fadel and Deutch, 2002), is strongly associated with cue-reinstated drug and food-seeking behaviours (Harris *et al.*, 2005). Second, intra-VTA injection of orexin-A induced reward-seeking behaviours in extinguished rodents in an OX_1 receptor-dependent manner (Mahler *et al.*, 2012). Third, systemic or intra-VTA injection of an antagonist of OX_1, but not OX_2, receptors significantly reduced the reinstatement of extinguished seeking behaviours for cocaine, alcohol or morphine elicited by drug-predicting cues or yohimbine (Mahler *et al.*, 2012), but not foot shock stress (Wang *et al.*, 2009). Finally, OX_1 receptor activation and AMPA transmission in VTA are simultaneously necessary for cues, but not cocaine, to trigger drug seeking (Mahler *et al.*, 2013). It remains to be elucidated by *in vivo* studies if endogenous orexins released during the reinstatement induced by stress will increase VTA dopaminergic activity through a glutamate-receptor-mediated mechanism.

Chiou and colleagues further demonstrated that endocannabinoid-mediated disinhibition of dopaminergic neurons contributes to acute restraint stress-induced reinstatement of extinguished cocaine-seeking behaviour in mice (unpublished observations presented at the 2013 INRC meeting; Chiou *et al.*, 2013). A 30 min restraint stress, which increased orexin-A levels in the VTA and Fos activation in LH orexin neurons, significantly reinstated extinguished cocaine CPP in mice. This acute stress-induced cocaine reinstatement was absent in CB_1 receptor-knockout mice, and was prevented by intra-VTA injection of antagonists for OX_1, CB_1 or a DAGL inhibitor. Several previous studies have suggested that endocannabinoids are involved in the reinstatement of extinguished seeking behaviours for drugs of abuse. Indeed, cue- or swim-stress-induced seeking behaviours for cocaine (De Vries *et al.*, 2001), heroin (Fattore *et al.*, 2003), ethanol (Cippitelli *et al.*, 2005) or nicotine (De Vries *et al.*, 2005) were blocked by a CB_1 receptor antagonist. Therefore, one could hypothesize that acute restraint stress activates hypothalamic orexin neurons, likely leading to downstream release of orexin in the VTA and the activation of OX_1 receptors on dopaminergic neurons that causes an endocannabinoid-mediated retrograde inhibition of GABA release, resulting in disinhibition of VTA dopaminergic neurons and the initiation of cocaine-seeking behaviour.

Orexin receptor signalling at ascending projection targets on ethanol self-administration, ethanol seeking and stress-induced relapse

Alcohol dependence constitutes a major global public health problem and there remains a critical need for the development of medications for the treatment of alcohol use disorders. There is an extensive literature demonstrating that activation of orexin-containing neurons and orexin receptors modulate alcohol consumption and cue-induced relapse (see Kim *et al.* 2012). While their mechanism of action remains unclear, these receptors may be promising targets for the treatment of alcohol use disorders.

The Lawrence laboratory was the first to demonstrate a role for orexins in both ethanol consumption and cue-induced reinstatement of ethanol seeking in rats (Lawrence *et al.*, 2006). These effects were specific, with a differential effect of OX_1 receptor antagonism on the motivational strength of ethanol compared with sucrose (Jupp *et al.*, 2011a). A feature of human addiction is an enduring propensity to relapse, which can be modelled in rodents for potential translational relevance. After long-term ethanol self-administration, rats were subjected to extinction training and

then tested for cue-induced reinstatement of ethanol seeking either immediately after extinction or when extinction was followed by protracted abstinence (5 months). Representation of cues previously paired with ethanol availability was sufficient to precipitate reinstatement of ethanol seeking at both time points. Pretreatment with the OX_1 receptor antagonist, SB 334867, effectively prevented cue-induced reinstatement of ethanol seeking both immediately after extinction and also after protracted abstinence (Jupp *et al.*, 2011b). These data therefore provide evidence that cue-conditioned ethanol seeking involves the release of endogenous orexins acting upon OX_1 receptors. Indeed, these findings are consistent with a role for orexins in the integration of cue salience, even after long-term withdrawal. While the VTA is heavily implicated as a site for orexin-mediated modulation of reward seeking (Harris *et al.*, 2005; Borgland *et al.*, 2006), Lawrence and colleagues sought to examine the potential for other regions to contribute to this behaviour. The prefrontal cortex (prelimbic and orbitofrontal) was identified by Fos expression as a potential locus where ascending orexinergic input could modulate cue-conditioned ethanol seeking. Thus, OX_1 receptor antagonism attenuated reinstatement-induced Fos expression in prelimbic and orbitofrontal cortices (Jupp *et al.*, 2011b). Therefore, in addition to acting within the VTA, ascending orexin projections may also be implicated in cue-driven ethanol seeking (Brown and Lawrence, 2013).

Orexins in the amygdala and stress-induced reinstatement of ethanol seeking and relapse

The orexin system plays a role in response to stress and orexin neurons are activated in response to arousal, which is a key component of the stress response system (Winsky-Sommerer *et al.*, 2004; Boutrel *et al.*, 2005; Richards *et al.*, 2008). Orexins have been implicated in the post-stress anxiogenic behaviour in rats exposed to foot shock treatment, and antagonism of orexin receptors decreases not only anxiety (Chen *et al.*, 2013b) but also blood pressure in spontaneously hypertensive rats (Li *et al.*, 2013). The extended amygdala plays a critical role in the reinstatement of drug seeking, as inactivation of the central nucleus of the amygdala (CeA) prevents foot shock-induced reinstatement of cocaine seeking (McFarland *et al.*, 2004). Projections from orexin neurons densely innervate the CeA (Peyron *et al.*, 1998; Schmitt *et al.*, 2012), a brain region implicated in emotional behaviours such as anxiety, fear and stress (Lin *et al.*, 1999). A previous study had shown that in acute rat brain slices, orexins enhance firing of neurons of the central medial nucleus of the CeA (Bisetti *et al.*, 2006). The central and medial amygdaloid projections regulate the hypothalamic-pituitary-adrenal axis and innervate orexin-containing neurons in the LH (Gray *et al.*, 1989; Dayas *et al.*, 1999; Nakamura *et al.*, 2009). Furthermore, hypothalamic orexin neurons have reciprocal projections to the amygdala and play a role in vigilance and stress-related responses (Nishino, 2011). The OX_1 receptor antagonist, GSK1059865, can inhibit the yohimbine (pharmacological stressor) activated extra-hypothalamic stress neurocircuits in the extended amygdala (Gozzi *et al.*, 2013). Furthermore, the dual OX_1 and OX_2 receptor antagonist, almorexant, decreases fear-potentiated startle responses in rats, which is a model of conditioned fear involving the CeA (Steiner *et al.*, 2012). Additionally, orexin receptor antagonists reduce panic responses induced by anxiogenic drugs (Johnson *et al.*, 2012). Together, this suggests that orexins in the amygdala play a role in stress responses, including those underlying drug relapse.

Orexins in the mPFC: role in ethanol seeking and stress-induced relapse

The mPFC has been known to regulate seeking behaviour for most drugs of abuse including cocaine (Pietro *et al.*, 2008; Pentkowski *et al.*, 2010; Chen *et al.*, 2013a; Cisler *et al.*, 2013) and ethanol (George *et al.*, 2001; Samson and Chappell, 2003). The mPFC, VTA and the NAc core have been proposed to interact in a dopamine-dependent manner to influence the behavioural aspects of ethanol seeking (Groenewegen *et al.*, 1990; Cador, 1992; Yang *et al.*, 1999). Most importantly, ambient environmental cues coupled with endogenous stimuli (level of stress, anxiety, dysphoria) are processed at the level of mPFC (Kalivas and Klitenick, 1993) to trigger alcohol-seeking behaviour. Depending on the rewarding salience of the stimuli, the mPFC causes an increase in the dopamine levels in the NAc core and VTA through glutamatergic innervation (Sesack *et al.*, 2003; Geisler *et al.*, 2007) in conjunction to the direct excitatory effects of ethanol on VTA dopaminergic neurons (Brodie *et al.*, 1999). An ethanol-induced increase in dopamine affects the NAc core and mPFC so as to sustain the rewarding salience of the initial stimuli (i.e. self-administration) with a concomitant decrease in excitatory inputs from the mPFC (Seamans *et al.*, 2001).

The mPFC-induced modulation of VTA dopaminergic neurons through glutamate transmission is enhanced by orexins particularly during the active/wake period (Moorman and Aston-Jones, 2010). The rewarding salience of the external stimuli and direct orexinergic innervation of VTA dopaminergic neurons are thought to strengthen the mPFC-VTA dopamine connection (Moorman and Aston-Jones, 2010) that drives bias towards reward-seeking and goal-directed responses. This role of orexin aligns perfectly with its ability to potentiate glutamate transmission in VTA dopaminergic neurons in cocaine-induced synaptic plasticity (Borgland *et al.*, 2006). Orexin-A has also been shown to excite pyramidal cells in the mPFC (layers II–VI) (Yan *et al.*, 2012) to strengthen intracortical connections and facilitate information processing between mPFC and other parts of the prefrontal cortex to achieve appropriate cognitive and reward-related responses. Stressful stimuli and stress-induced adaptations disrupt normal signalling in the PFC (Liu and Aghajanian, 2008; Caffino *et al.*, 2013). Exposure to stress in cocaine-habituated rats causes dysregulation in glutamate neurotransmission in the mPFC at synaptic clefts leading to reduced glutamate reuptake and sensitization of glutamate neurons to stress (Caffino *et al.*, 2013). These changes may be subjectively experienced as stressful or dysphoric and presumably manifest in a 'depression-like' state that may lead to stress-induced drug reinstatement. Orexins have been previously implicated in stress-induced alcohol reinstatement and the activity of excitatory orexin currents in the mPFC has been shown to be partially affected under stressful conditions (Liu and Aghajanian, 2008). This suggests that orexin transmission in the mPFC could exacerbate a stress-induced negative emotional state by decreasing arousal with a

concomitant urge to mitigate the negative affective state by using drugs. As orexin receptor antagonists are optimized and reach the market for other medical indications, their use as part of a multifaceted approach in the treatment of addiction is indeed promising.

Effects of co-release of orexin and dynorphin on reward and drug seeking

While expression of orexin peptides is confined to several thousand cells in the LH, dynorphin and its cognate, κ-opioid, receptor are found more widely across the neuraxis (Chavkin *et al.*, 1982; Mansour *et al.*, 1994). Although orexin does appear to play an important role in the heightened arousal that accompanies stress (Winsky-Sommerer *et al.*, 2004), the balance of evidence suggests that dynorphin release and κ-opioid receptor activation are responsible for the aversive components of stress (Van't Veer and Carlezon, 2013). For example, dynorphin and κ-opioid receptor expression are altered predictably in the NAc, amygdala and several other brain structures in animals subjected to stress (Shirayama *et al.*, 2004; Knoll *et al.*, 2011); central or peripheral administration of κ-opioid receptor agonists causes conditioned place aversions in rodents (Bals-Kubik *et al.*, 1993), and produces anxiety and dysphoria in humans (Pfeiffer *et al.*, 1986; Walsh *et al.*, 2001); and the aversive effects of 'stress peptides' like corticotropin-releasing factor (CRF) exert their dysphoric effects via dynorphin and κ-opioid receptor activation (Land *et al.*, 2008). Indeed, dynorphin- κ-opioid receptor signalling has been conceptualized to work in concert with CRF as part of a 'brain anti-reward system' that serves to offset the function of natural reward circuitry of which the orexin system may be a part (Koob and Le Moal, 2008).

Counter-intuitively, almost all (~95%) orexin neurons in rats and mice express dynorphin (Chou *et al.*, 2001). This pattern of expression is conserved in humans (Crocker *et al.*, 2005), and *in vitro* data support co-release of orexin and dynorphin (Li and van den Pol, 2006), suggesting functional relevance. Interestingly, mRNA for both prepro-orexin and preprodynorphin increases after acute cocaine abstinence in rats exposed to chronic escalating doses of cocaine (Zhou *et al.*, 2008), suggesting that orexin and dynorphin release may be sensitive to cocaine exposure and/or acute withdrawal. In contrast, withdrawal from morphine does not alter preprodynorphin mRNA (Zhou *et al.*, 2006).

At this time, potential functions of this pattern of gene expression can only be speculated, but the presence of an excitatory (orexin) and inhibitory neuropeptide (dynorphin) in the same neuron suggests a number of possibilities. The first is a simple negative feedback mechanism whereby dynorphin can hyperpolarize orexin-dynorphin containing terminals and reduce the probability of transmitter release under conditions of prolonged excitation. Because many orexin-containing neurons are glutamatergic (Rosin *et al.*, 2003), this motif may be important for modulating a potent source of excitatory output onto target neurons, thus preventing hyperexcitability in both target neurons and the orexin-dynorphin neurons themselves. Another possibility permits robust gain control of target neurons given differential expression of OX_1 and κ-opioid receptors in different brain structures or nuclei. Feedforward disinhibition of target neurons by the expression of κ-opioid receptors on GABAergic interneurons may occur in parallel with direct excitation of OX_1 receptor-expressing target neurons. This type of synaptic arrangement would facilitate rapid increases in firing rate and an accompanying transient spike in transmitter released by target neurons. This mechanism could, for instance, induce the type of burst firing and forebrain phasic dopamine release known to be important for reward learning (Schultz, 2002; Tsai *et al.*, 2009; Steinberg *et al.*, 2013).

These ideas remain untested; however, Muschamp and colleagues have used a combination of behavioural, cellular and molecular approaches to demonstrate the importance of orexin-dynorphin co-expression both on functioning of VTA dopaminergic neurons and some of the reward-associated behaviours they control. Preliminary data presented at the 2013 INRC meeting suggest that acute actions of orexin do not mediate reward *per se*. Rather, orexin appears to facilitate reward by occluding the 'anti-reward' effects of its co-transmitter dynorphin (Muschamp *et al.*, 2012).

Summary

Dopaminergic neurons in the VTA are a key target of addictive drugs and neuroplasticity in this region may underlie some of the core features of addiction. Orexin neurons are activated by abused drugs, stress and cues predicting drugs or stress. Abused drugs can also induce synaptic plasticity of orexin neurons. However, this alone is not sufficient to drive drug-seeking behaviour. Indeed, orexin action in the VTA exerts modulatory effects on a variety of behaviours produced by drugs of abuse. Mechanisms underlying orexin-mediated effects in the VTA on drug-seeking behaviour have been proposed. Acute application of orexin potentiates excitatory synaptic transmission in the VTA, and inhibition of OX_1 receptor signalling blocks both cocaine- or morphine-induced plasticity. Additionally, it has been proposed that orexins can induce an endocannabinoid-mediated retrograde inhibition of GABA release, resulting in a disinhibition of dopamine neurons. Orexins may also promote alcohol seeking as well as cue- or stress-induced relapse at sites outside the VTA. OX_1 receptor antagonists blocked cue-induced ethanol seeking as well as Fos expression in the medial and orbitoprefrontal cortices. Furthermore, OX_1 receptor signalling in the CeA as well as the mPFC has also been implicated in stress-induced relapse to ethanol seeking. These studies suggest that the ascending orexin projections may also play a critical role in alcohol-seeking behaviours. It will be important to determine if these sites are also involved in addiction-related behaviour for other drugs of abuse, including morphine. Finally, excitatory orexins are co-expressed and potentially co-released with the aversion-inducing neuropeptide, dynorphin. Opposing effects of these peptides have been proposed to facilitate reward by orexin-mediated occlusion of the 'anti-reward' effects of dynorphin. Taken together, the development of novel OX_1 receptor antagonists could have excellent utility in the treatment of drug craving and relapse.

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Conflict of interest

None.

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