The orexin system and nicotine addiction: preclinical insights

Short title: Orexins and nicotine

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Abstract

Current pharmacotherapies for smoking have only modest efficacy with failure rates of up to 90%. One potential target for new pharmacotherapies is the orexin/hypocretin system, a hypothalamic neuropeptide system involved in arousal, appetite and reward. The orexin system has been suggested as a potential therapeutic target for nicotine addiction because there are orexin/nicotine interactions following both acute and chronic nicotine administration, preclinical findings in animal models of nicotine addiction and some correlational studies in humans. Acute orexin administration activates cholinergic neurons and acute nicotine administration activates orexin neurons, while chronic nicotine causes an upregulation in orexin peptides and receptors while decreasing the availability of orexin binding sites. A small number of preclinical studies in animal models of nicotine addiction have found effects of orexin antagonists in reducing nicotine self-administration and/or reinstatement behavior, but results are not always entirely consistent between studies. A few human studies have found negative correlations between orexin levels and measures of nicotine craving in humans, or genetic associations between hypocretin receptor polymorphism and nicotine dependence. However, further research is required to reconcile discrepancies in the preclinical literature and to understand the role of the orexin system in nicotine dependence before clinical trials can be proposed.

Definitions

Almorexant. A dual orexin receptor antagonist that progressed to clinical trials.

Dual orexin receptor antagonist. A compound that has antagonist effects at both orexin receptors.

Fixed-ratio self-administration. Operant reinforcement schedule used in preclinical animal models where a reward is earned for a fixed number of operant responses.

Hypocretin. The orexin/hypocretin system named based on its *hypo*thalamic distribution and similarity to the in*cretin* family of hormones. Refers to the genes HCRT/*Hcrt*, which encodes for the two peptides, HCRTR1/*Hcrtr1* and HCRTR2/*Hcrtr2* which encode for the two receptors respectively.

Orexin. The orexin/hypocretin system named based on its role in appetitive behaviour. Refers to the two peptides, orexin-A and orexin-B, which bind to the OX_1 and OX_2 receptors.

Progressive ratio. Operant reinforcement schedule used in preclinical animal models where the number of responses required to earn each subsequent reward increases.

Reinstatement. A model of relapse-like behaviour which involves the return of a previously extinguished behaviour. May be precipitated by drug-priming, reward-associated cues, contexts or stress.

SB-334867. A selective OX_1 receptor antagonist.

SORA-18. A selective OX_2 receptor antagonist.

TCS-OX2-29. A selective OX_2 receptor antagonist.

TCS 1102. A dual orexin receptor antagonist.

Key Facts of the Orexin System

- Discovered simultaneously by two groups in 1998.
- Two peptides encoded by the hypocretin gene, orexin-A and orexin-B, bind to excitatory G-protein coupled receptors, OX_1 and OX_2.
- Loss of orexin neurons involved in human narcolepsy.
- Stimulation of orexin system promotes appetitive behaviour.
- Animal studies have demonstrated roles for the orexin in multiple drugs of abuse.
- Suvorexant (Belsomra®) was the first orexin-based pharmacotherapy and was approved in 2014 for the treatment of insomnia.

Summary Points

- This chapter focuses on the orexin system, a neuropeptide system involved in arousal, appetite and reward, and its potential as a therapeutic target for nicotine addiction.
- The orexin system activates cholinergic neurons and is activated by nicotine administration, with chronic nicotine causing altered expression of the hypocretin genes and orexin peptides.
- A small number of preclinical animal studies have shown effects of various orexin receptor antagonists in self-administration or reinstatement paradigms.
- However, there are substantial differences in methodologies between studies and some studies have not found effects of orexin antagonism.
- The limited number of studies in animals and humans suggests that there may be some promise in targeting the orexin system, but more research is required to understand its role in nicotine seeking before clinical trials can be proposed.

Key words: orexin, hypocretin, self-administration, reinstatement, SB-334867, TCS 1102

List of abbreviations

***Acb*.** Nucleus accumbens

***DMH*.** Dorsomedial hypothalamus

***FRn*.** Fixed ratio of n responses

***GPCR*.** G-protein coupled receptor

***LH*.** Lateral hypothalamus

***nAChR*.** Nicotinic acetylcholine receptor

***OX-A*.** Orexin-A

***OX-B*.** Orexin-B

***PeF*.** Perifornical hypothalamus

***PFC*.** Prefrontal cortex

***PR*.** Progressive ratio

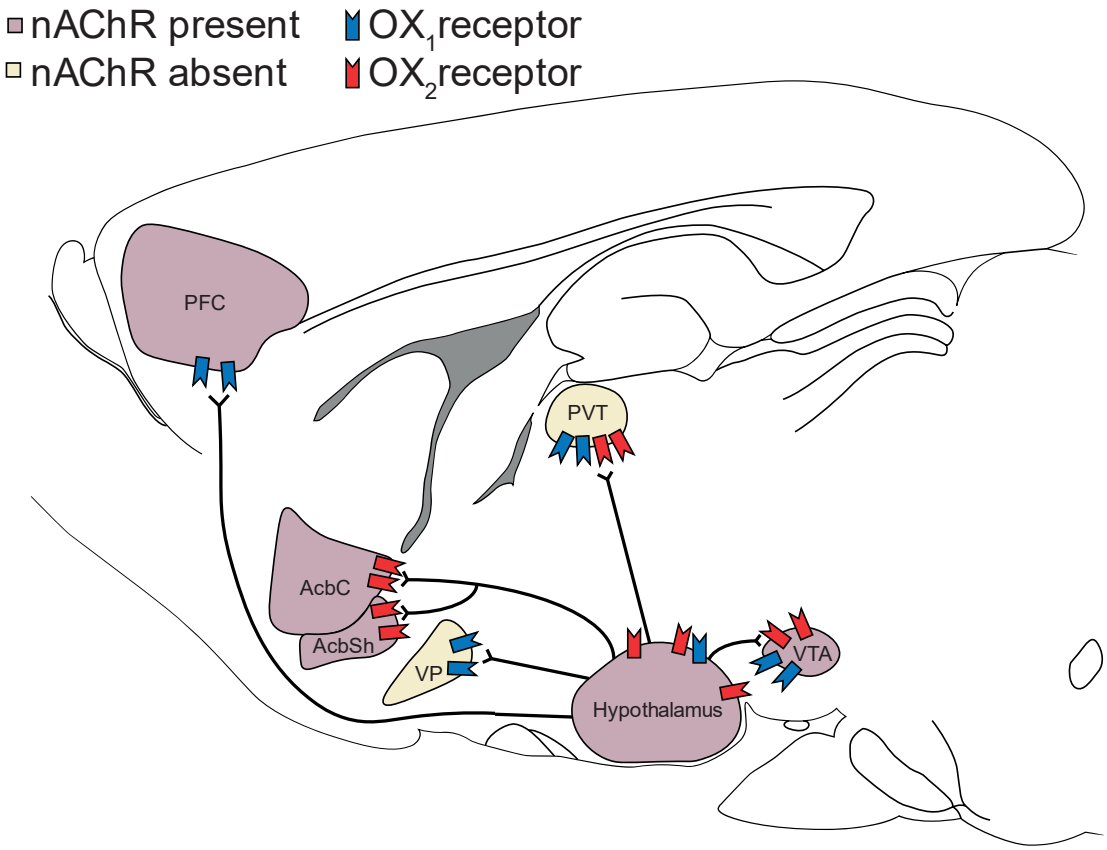
***VTA*.** Ventral tegmental area

Current pharmacological therapies for smoking have only modest efficacy with failure rates of up to 90% (Cahill, Lindson-Hawley, Thomas, Fanshawe, & Lancaster, 2016). The orexin/hypocretin system has been suggested as a potential therapeutic target and is in clinical trials for cocaine (The University of Texas Health Science Center, 2016). Here we review the orexins as a potential therapeutic target for nicotine because there is evidence for both acute and chronic nicotine/orexin interactions and some preliminary evidence that orexin-based medications may be effective in reducing smoking. However, while there is promise there are some conflicting preclinical results and only correlational results in human studies are available.

The orexin/hypocretin system is a neuropeptide system involved in arousal, appetite, and reward. Orexin neurons originate exclusively from the lateral hypothalamus (LH), perifornical hypothalamus (PeF) and dorsomedial hypothalamus (DMH; Baldo, Daniel, Berridge, & Kelley, 2003; Elias et al., 1998; Peyron et al., 1998). Orexin fibres project to key mesocorticolimbic reward regions (Figure 1), such as the ventral tegmental area (VTA), nucleus accumbens (Acb) and prefrontal cortex (PFC; Baldo et al., 2003; Peyron et al., 1998) where there are abundant nicotinic acetylcholine receptors (nAChRs; Lerman et al., 2007).

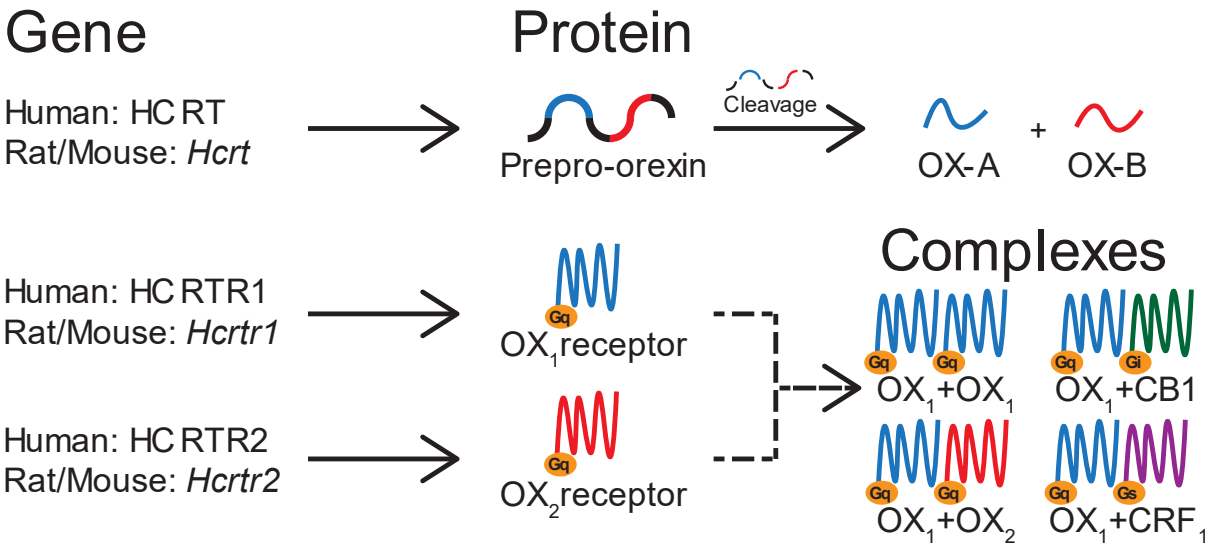
The orexin system is composed of two peptides, orexin-A and orexin-B (OX-A and OX-B respectively), and two GPCRs, OX_1 and OX_2 (Figure 2; Sakurai et al., 1998). Hypocretin (Human: HCRT, Rat/Mouse: *Hcrt*) mRNA encodes for the prepro-orexin precursor peptide which is cleaved to form the OX-A and OX-B peptides (Sakurai et al., 1999). OX-A binds non-selectively to both receptors while OX-B shows some selectivity for the OX_2 receptor. Both OX_1 and OX_2 are excitatory and produce elevated Ca^{2+} suggesting $G\alpha_q$-mediated signaling (de Lecea et al., 1998; Sakurai et al., 1998) but they can also interact with multiple second messenger pathways (Kukkonen & Leonard, 2014). Orexin receptors also form complexes, which may be homodimers (Xu, Ward, Pediani, & Milligan, 2011), OX_1 and OX_2 heteromers,

Figure 1: Anatomic overlap between the orexin system and nicotinic acetylcholine receptors.



Legend to Figure 1: Orexin receptors are present in varying densities in key brain regions involved in reward. Nicotinic acetylcholine receptors (nAChRs) are present in all of these areas, except for the ventral pallidum (VP) and paraventricular nucleus of the thalamus (PVT).

Figure 2: Schematic overview of orexin signalling molecules.



Legend to Figure 2: The two orexin peptides are cleaved from a single precursor and bind to the orexin receptors, which may exist as complexes with each other, CB1 or CRF_1 receptors.

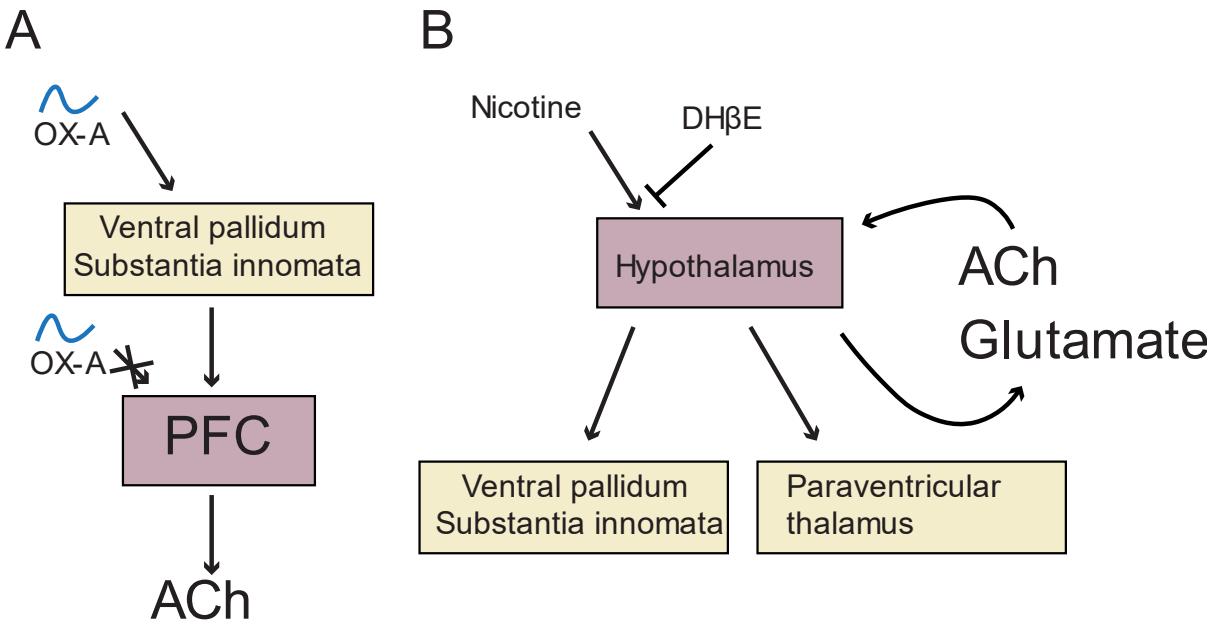
CB_1 or CRF receptor complexes (Ellis, Pediani, Canals, Milasta, & Milligan, 2006; Jäntti, Mandrika, & Kukkonen, 2014; Navarro et al., 2015). Thus, orexin signaling is thought to be excitatory with a $G\alpha_q$-mediated signaling cascade, but with the potential for interactions with co-transmitters and heteromeric receptor complexes.

Neuroanatomical and Molecular Interactions

Acute Nicotine and Orexin

The orexin system has been suggested as a potential therapeutic target because of the neuroanatomical and molecular interactions between the cholinergic and orexinsystems. Interactions occur following both acute and chronic nicotine administration. Cholinergic neurons are activated by OX-A application (Fadel, Pasumarthi, & Reznikov, 2005) and orexin neurons are activated by acute systemic nicotine administration (Figure 3; Pasumarthi, Reznikov, & Fadel, 2006). Importantly, this activation, as measured by c-Fos immunohistochemistry, appears to be specific in the hypothalamus for orexin neurons, suggesting that the orexins play a key role in nicotine-mediated effects. While this effect is blocked by both the non-selective nAChR antagonist mecamylamine and the α4β2 nAChR antagonist DHβE, DHβE alone increased medial hypothalamic orexin neuron activation, but blocked nicotine-induced activation (Pasumarthi et al., 2006), suggesting that there is endogenous cholinergic regulation of the orexin system. Nicotine-induced activation of orexin neurons appears to be specific to hypothalamic projections to the basal forebrain and paraventricular thalamus (Pasumarthi & Fadel, 2008). In addition to systemic nicotine administration, orexin neurons can also be activated by local nicotine application to the hypothalamus which produces increased ACh and glutamate efflux in the hypothalamus (Pasumarthi & Fadel, 2010). These results demonstrate interactions between orexin, which

Figure 3: Acute orexin/nicotine interactions.



Legend to Figure 3: (A) Acute orexin can activate cholinergic neurons and (B) acute nicotine can activate orexin outputs and result in local glutamate and ACh release.

activates cholinergic neurons, and acute nicotine administered systemically or directly to the hypothalamus that activates orexin neurons.

Chronic Nicotine and Orexin

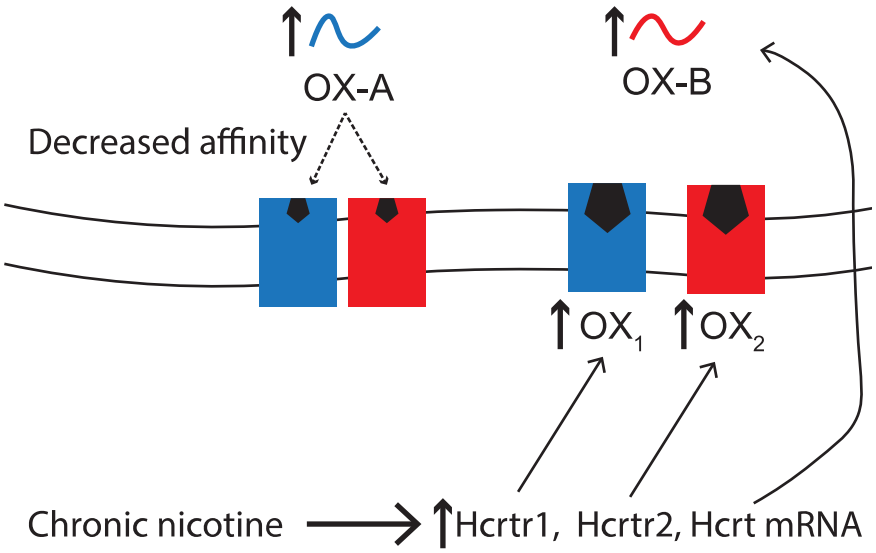
Studies of chronic nicotine administration have shown alterations in regulation of hypocretin gene expression and orexin peptides (Figure 4). Chronic nicotine administration (14 days) increases levels of mRNA encoding the prepro-orexin peptide and orexin receptors (Kane et al., 2000). Blockade of nAChRs in nicotine-exposed rats decreases orexin neuron activation (Simmons et al., 2016). Measurements of protein show that in the DMH, chronic nicotine increase both OX-A and OX-B, while OX-B is elevated in the paraventricular nucleus (Kane et al., 2000). However, chronic nicotine also decreases hypothalamic bindings sites available for OX-A (Kane, Parker, & Li, 2001). This desensitization appears to occur by obscuring binding sites but without internalization because OX-A continued to bind, but with lower affinity (Kane et al., 2001). Although a more direct demonstration of the exact mechanism of nicotine-induced decrease in binding sites has never been reported in the literature, these results demonstrate that chronic nicotine administration has regulatory effects on both hypocretin gene expression and levels of orexin peptide.

Orexin as a Therapeutic Target for Nicotine Dependence

Nicotine Self-Administration

Preclinical studies of orexin as a potential therapeutic target for nicotine addiction have found effects of both selective and non-selective (dual) orexin receptor antagonists (Table 1). The first direct demonstration of a role for orexins in nicotine seeking behaviour involved administration of the OX_1 receptor antagonist SB-334867 systemically and into the insular cortex (Hollander, Lu, Cameron, Kamenecka, & Kenny, 2008). OX_1 receptor antagonism selectively suppressed the number of nicotine infusions earned on fixed-ratio-5 (FR5) and

Figure 4: Effects of chronic nicotine on orexin regulation.



Legend to Figure 4. Chronic nicotine upregulates expression of orexin peptides and receptors while but reduces the availability of binding sites for OX-A without receptor internalisation.

Table 1: Comparison of behavioural studies examining nicotine self-administration and reinstatement.

Animals	Food Restriction	Pretraining	Training	Measure	Drug	Outcome	Reference
Pair-housed male Wistars	To 85% bodyweight	Food FR5TO20 until stable	Lever, 7 – 14 days	FR5TO20	SB-334867 (i.p.)	↓	Hollander et al. (2008)
				PR	SB-334867 (i.p.)	↓	
				FR5TO20	SB-334867 (intra-insula)	↓	
Singly-housed male Long-Evans rats	18 g/day	Food until 100 pellets/1 h	Lever, 40 ± 4.5 days	FR5TO60	SB-334867 (i.p.)	↓	LeSage et al. (2010)
			66 ± 8.1 days	FR5TO60	Almorexant (i.p.)	↓	
Singly-housed male C57BL/6J mice	*Ad libitum*	None	Daily nicotine prime, nosepoke, 10 days	OX-A-induced reinstatement (i.c.v.)	SB-334867 (i.p.)	↓	Plaza-Zabala et al. (2010)
				Footshock reinstatement	SB-334867 (i.p.)	No effect	
Singly-housed male C57BL/6J mice	*Ad libitum*	None	Daily nicotine prime, nosepoke, 10 days	Cue reinstatement	SB-334867 (i.p.)	↓	Plaza-Zabala et al. (2013)
					TCS-OX2-29 (i.p.)	No effect	
Singly-housed male Long-Evans rats	25 g/day	Food FR1	Lever, FR1 x 5 days, FR2 x 2 days, FR5 x 6 days	PR	2-SORA 18 (p.o.)	No effect	Uslaner et al. (2014)
			Animals retrained FR5 x 3 days	Cue reinstatement	2-SORA 18 (p.o.)	↓	
				Nic reinstatement	2-SORA 18 (p.o.)	No effect	
Group-housed Sprague Dawley rats	20 g/day	None	Nosepoke, 10 days	FR1TO24	TCS 1102 (i.c.v.)	No effect	Khoo et al. (2017)
			Animals retrained (Total 29 days self-admin)	Cue reinstatement	TCS 1102 (i.c.v.)	No effect	
				Nic reinstatement	TCS 1102 (i.c.v.)	No effect	
				Compound reinstatement	TCS 1102 (i.c.v.)	No effect	

Legend to Table 1: Details of key parameters of preclinical studies and the direction of effect.

progressive ratio (PR) schedules, while leaving the number of food pellets earned unaffected. Similarly, intra-insular administration of SB-334867 reduced FR5 nicotine infusions but not the number of food pellets earned (Hollander et al., 2008). These results have been replicated using SB-334867 and the dual orexin receptor antagonist almorexant (LeSage, Perry, Kotz, Shelley, & Corrigall, 2010). The number of nicotine infusions earned was reduced on an FR5 schedule by both the OX_1 receptor antagonist SB-334867 and the dual orexin receptor antagonist almorexant. However, while SB-334867 in this study also left the number of food pellets earned unaffected, the highest dose of almorexant reduced pellets earned. LeSage et al. (2010) further extended the findings of Hollander et al. (2008) by examining *Hcrtr1* and *Hcrtr2* gene expression and finding decreased *Hcrtr1* in the rostral LH immediately after nicotine self-administration but not 5 h after a self-administration session, while arcuate nucleus had increased *Hcrtr1* mRNA 5 h but not immediately after self-administration. These consistent results from different groups provided strong indications that the orexins might be a potential target for the treatment of nicotine addiction.

Reinstatement of Nicotine Seeking

In addition to regulating self-administration of nicotine, it has also been shown that orexin signaling plays a role in the reinstatement of nicotine seeking but results have not been consistent. Intracerebroventricular administration of OX-A can reinstate extinguished nicotine seeking behaviour in mice, an effect that can be blocked by administration of the OX_1 receptor antagonist SB-334867 (Plaza-Zabala, Martín-García, de Lecea, Maldonado, & Berrendero, 2010). While OX_1 receptor antagonism reduces anxiety-like behaviour in the elevated plus maze induced by acute injections of nicotine, suggesting that orexins are involved in the anxiogenic effects of acute nicotine OX_1 receptor antagonism does not affect footshock-induced reinstatement, indicating that these behaviours are mediated by separate processes (Plaza-Zabala et al., 2010). During cue-induced reinstatement of nicotine-seeking in mice, an

increased percentage of orexin neurons in the PeF and LH show signs of activation as measured by c-Fos (Plaza-Zabala et al., 2013). Selective OX_1 receptor antagonist using SB-334867 but not OX_2 receptor antagonism using TCS-OX2-29 can attenuate this cue-induced reinstatement (Plaza-Zabala et al., 2013). However, it has been shown that a different selective OX_2 receptor antagonist (SORA-18) was able to reduce cue-induced reinstatement in rats, but not nicotine-primed reinstatement or PR self-administration (Uslaner et al., 2014). These studies suggest that the role of orexin signaling in reinstatement behaviours is inconsistent and may depend on the proximal cause of reinstatement or the behavioural model used. In both rats and mice, cue-induced reinstatement has been reduced using orexin receptor antagonists, but it is unclear whether the OX_2 receptor is involved. While these results are suggestive of a role for the orexins in cue-induced reinstatement for nicotine, the relatively small number of studies and the variance in training protocols (Table 1) makes it difficult to draw strong conclusions.

Withdrawal and Motivation

Preclinical studies have also demonstrated roles for orexin signaling in other nicotine-related behaviours. In mice made dependent by 25 mg/kg/day nicotine administered for 14 days by osmotic minipump, mecamylamine-precipitated withdrawal was attenuated in *Hcrt*$^{-/-}$ mice or mice given the OX_1 receptor antagonist SB-334867 (Plaza-Zabala, Flores, Maldonado, & Berrendero, 2012), suggesting that orexins mediate withdrawal symptoms. Orexin signaling in the paraventricular nucleus of the hypothalamus was particularly implicated because targeted microinjection of SB-334867 attenuated behavioural signs of nicotine-withdrawal. Although Plaza-Zabala et al. (2012) did not find effects of the OX_2 receptor antagonist TCS-OX2-29, this may have been because they did not use a sufficiently high dose. Their doses of 5-10 mg/kg TCS-OX2-29 (Plaza-Zabala et al., 2012; Plaza-Zabala et al., 2013) were lower than the 10-30 mg/kg doses used by Smith, See, and Aston-Jones (2009) although these also produced null results for cocaine. A previous study that reported positive effects at OX_2 receptors used a 15

mg/kg dose of SORA-18, which has greater potency than TCS-OX2-29 (Hirose et al., 2003; Uslaner et al., 2014). The dual orexin receptor antagonist, TCS 1102, also attenuated nicotine-potentiated PR responding and nicotine-induced reinstatement for sucrose pellets (Winrow et al., 2010). These results also provide support for a potential use for orexin receptor antagonists in treating nicotine addiction because they might reduce symptoms of withdrawal or reduce other reward-related nicotine responses.

Divergent Findings

However, we recently reported that the dual orexin receptor antagonist TCS 1102 had no effect on nicotine self-administration, cue-induced reinstatement, nicotine-primed reinstatement and had at most a small transient effect on cue and prime-induced reinstatement only after chronic nicotine self-administration (Khoo, McNally, & Clemens, 2017). Although TCS 1102 has previously been shown to be effective in reducing behavioural responses to nicotine (Winrow et al., 2010) and we found that intracerebroventricular TCS 1102 could attenuate OX-A-induced increases in feeding behaviour, we found no evidence of any effect of dual orexin receptor antagonism that could have clinical relevance. We suspect that this discrepancy between our study and previous findings may be due to several differences in protocols. For example, our rats were trained on FR1, while previous studies have generally used FR5 schedules of reinforcement and food pre-training protocols (Hollander et al., 2008; LeSage et al., 2010; Uslaner et al., 2014). In our study, rats were trained to make nosepokes for nicotine for the entire duration of training with no period of operant responding for food (Khoo et al., 2017). This may have resulted in relatively lower levels of motivation in our animals. It has previously been argued that nicotine has relatively weak primary reinforcing properties but may act to enhance the motivational properties of other reinforcers and conditioned stimuli (Chaudhri et al., 2006). If this is the case, the food pre-training protocols used in previous demonstrations of orexinergic regulation of nicotine seeking or self-

administration may have potentiated the motivational properties of the conditioned reinforcers and responses that were ultimately paired with nicotine. It has previously been shown that food pre-training enhances acquisition (Bongiovanni & See, 2008; Clemens, Caillé, & Cador, 2010; Garcia, Lê, & Tyndale, 2014). For example, sucrose pre-training can facilitate later cue-induced reinstatement of nicotine seeking for rats trained to make nosepokes (Clemens et al., 2010), but food pre-training does not appear to affect later reinstatement tests in rats trained to press levers (Garcia et al., 2014). Differences in pre-training protocols could also explain why relatively few rats in our experiments would self-administer under an FR5 schedule of reinforcement (unpublished observations).

These negative results urge caution in seeking to translate orexin antagonists to the clinic, but they do not necessarily exclude orexin-based therapies for nicotine. In humans, nicotine is often used in combination with other drugs (Cross, Lotfipour, & Leslie, 2017). For example, nicotine increases alcohol consumption in men (Acheson, Mahler, Chi, & de Wit, 2006; Barrett, Tichauer, Leyton, & Pihl, 2006) and subjective feelings of drunkenness (Kouri, McCarthy, Faust, & Lukas, 2004), results which have been replicated in animal models (Kalejaiye, Bhatti, Taylor, & Tizabi, 2013; Lê, Funk, Lo, & Coen, 2014). Nicotine use is also associated with using or becoming dependent on cannabis (Taylor et al., 2017), cocaine (Budney, Higgins, Hughes, & Bickel, 1993; Gorelick, Simmons, Carriero, & Tashkin, 1997) and methamphetamine (Grant et al., 2007). Using multiple drugs might increase the motivational salience and render nicotine subject to orexinergic regulation. Other drugs of abuse also have stronger evidence for orexinergic regulation and there is currently a clinical trial for using the dual orexin receptor antagonist, suvorexant, in the treatment of cocaine dependence (The University of Texas Health Science Center, 2016). However, further preclinical studies in animal models of polydrug use are required to establish whether this is the case.

Human Studies

A few studies in humans have found associations between the orexin system and nicotine (Table 2). Human studies have reported a negative correlation between orexin plasma concentration and self-reported nicotine craving (von der Goltz et al., 2010), and reduced *Hcrt* mRNA expression in blood samples in smokers compared to non-smokers (Rotter et al., 2012). Although these studies are consistent, they are limited by their peripheral measurement of orexin peptide or *Hcrt* mRNA. While the OX-A peptide can diffuse across the blood-brain barrier, OX-B has low lipophilicity and is rapidly metabolised (Kastin & Akerstrom, 1999). Genome-wide association studies have found that in Japanese samples, a single-nucleotide polymorphism in the HCRTR2 gene was associated with increased risk of smoking (Nishizawa et al., 2015). However, results from these studies should be interpreted with caution because it has been argued that genome-wide association studies produce little useful data when examining complex traits (Boyle, Li, & Pritchard, 2017), although previous studies have yielded associations between targets that are biologically relevant or already targets for current therapeutics (Visscher, Brown, McCarthy, & Yang, 2012). While Nishizawa et al. (2015) found associations between HCRTR2 polymorphism and smoking, methamphetamine and schizotypal trait scores, but they also found associations with goiter, aortic aneurysm and myeloma. The current small array of human studies of the orexin system and nicotine suggest that there may be some linkage, but this is correlational and further studies in animals and humans are required to better establish whether the orexin system really is involved in nicotine seeking.

Implications for Treatments

There is currently insufficient evidence to recommend orexin-based treatments for smoking. Although there are some promising preclinical results and correlational evidence has

Table 2: Human correlational studies.

Participants	Measurements	Findings	Reference
60 smokers, 64 non-smokers	Whole blood: plasma orexin Questionnaire of smoking urges Fagerström test for nicotine dependence	Smoking urges negatively correlated with plasma OX	(von der Goltz et al., 2010)
36 cannabis dependent, 20 smoking, 21 non-smoking student-acquaintances	Whole blood: HCRT and OX-A in peripheral blood lymphocytes	Lower OX-A in smokers No effect on HCRT promoter methylation	(Rotter et al., 2012)
Initial GWAS: 148 patients Followup: 112 abdominal surgery patients, 203 methamphetamine dependent patients, 311 healthy volunteers 2305 autopsy cases	Fagerström test for nicotine dependence Tobacco dependence screener Autopsy results	HCRTR2 single-nucleotide polymorphism (Val308Ile) correlated with smoking	(Nishizawa et al., 2015)

Legend to Table 2: Summary of key findings from human studies to date. GWAS, genome-wide association study.

been found in humans, there are also some inconsistent findings in the preclinical literature that should be addressed. Further studies are required to examine the relationship between an animal's training history and orexinergic regulation of nicotine seeking.

Conclusion

The orexin system has generated much excitement and interest for its therapeutic potential for a variety of disorders. This potential has recently been realised with the approval of suvorexant for insomnia (Coleman, Gotter, Herring, Winrow, & Renger, 2017) and may be realised for cocaine addiction (The University of Texas Health Science Center, 2016). Animal studies show that acute nicotine stimulates orexin neurons and chronic nicotine can have affect the regulation of the orexin system. Preclinical animal models of drug self-administration and seeking have tested orexin antagonists against nicotine less frequently than against other drugs of abuse, but from a relatively small pool of studies there is a mix of both positive and negative findings regarding the efficacy of orexin antagonists on nicotine self-administration and reinstatement. Research with human participants has found some consistent associations between orexin downregulation and nicotine use, as well as genetic associations between a single-nucleotide HCRTR2 polymorphism and smoking, but these results are correlational. For nicotine and orexin, there is currently insufficient evidence that it would be a useful therapeutic target for nicotine addiction but further studies are required to examine the reasons behind differential outcomes in preclinical research and to follow up on the associations that have been found in human studies.

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