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The orexin system regulates alcohol-seeking in rats

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1 Orexin-containing neurons have been implicated in feeding, sleep–wake cycles and more recently in drug-seeking behaviour.

2 Pretreatment of alcohol-preferring (iP) rats with an orexin$_1$ receptor antagonist (SB-334867, 20 mg kg^{-1}, intraperitoneally) completely abolished an olfactory cue-induced reinstatement of alcohol-seeking behaviour, and also attenuated alcohol responding under an operant fixed ratio regimen without affecting water responding.

3 The mRNA encoding orexin within the hypothalamus was expressed at a similar density in iP and non-preferring (NP) rats; chronic consumption of ethanol in iP rats did not significantly regulate the density of this expression, but did increase the area of expression within the lateral, but not medial, hypothalamus.

4 These data indicate that while orexin may not be implicated in the development of an alcohol preference, re-exposure of cues previously associated with alcohol availability is sufficient and adequate to activate orexin-containing neurons and drive reinstatement of alcohol-seeking.
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Abbreviations: CCK, cholecystokinin; CRF, corticotropin-releasing factor; CS$^+$, conditioned stimulus; DMSO, dimethylsulphoxide; FR3, fixed ratio 3; iP, inbred alcohol-preferring rat; NP, alcohol-nonpreferring rat; NPY, neuropeptide Y; S$^+$, unconditioned stimulus; s.e.m., standard error of the mean; VTA, ventral tegmental area

Introduction

Orexin (hypocretin)-containing neurons are confined to the hypothalamus (de Lecea *et al*., 1998; Peyron *et al*., 1998); however, these cells provide a widespread innervation of the neuraxis with orexinergic fibres. Cloning studies have indicated the presence of two main orexin peptides, namely orexin A and B, plus two main orexin receptors, namely orexin$_1$ and orexin$_2$ receptors (Sakurai *et al*., 1998). While initially implicated in the regulation of feeding (Sakurai *et al*., 1998), orexins also appear critically involved in vigilance, arousal and regulation of sleep–wake cycles (Chemelli *et al*., 1999; Lin *et al*., 1999; Hara *et al*., 2001). These latter behavioural facets also involve ascending noradrenergic systems originating in the locus coeruleus (A6 cell group). Notably, a considerable number of studies have implicated orexins/hypocretins with the regulation of A6 cell firing in this context (Hagan *et al*., 1999; Horvath *et al*., 1999b; Bourgin *et al*., 2000; Ivanov & Aston-Jones, 2000). Recently, the lateral hypothalamic orexin system has been implicated in reward to food, morphine or cocaine conditioning (Harris *et al*., 2005), raising the possibility of a novel therapeutic target for substance abuse and eating disorders. In addition, the orexin input to the ventral tegmental area (VTA) appears to play a role in cocaine-induced synaptic plasticity and behaviours associated with compulsive drug-seeking (Borgland *et al*., 2006).

Alcohol is one of the most widely used and abused substances in the world. While moderate alcohol intake may be generally beneficial, alcohol abuse causes as much, if not more death and disability as measles, malaria, tobacco or illegal drugs (World Health Organization, 2001). In economic terms, alcohol abuse has been estimated at US$167 billion per year; however, 'in human terms, the costs cannot be calculated' (National Institute on Alcohol Abuse and Alcoholism, 2004). For these reasons, there has been extensive research into the pathophysiology underlying alcoholism; however, current therapeutic approaches are far from adequate. This is largely owing to the polymodal nature of the disease and for this reason further research is required to identify new therapeutic targets for the improved treatment of alcoholism.

Drugs that interact with neuropeptide systems have great potential in the pharmacotherapy of alcoholism: witness the widespread use of the opioid antagonist naltrexone in the treatment of alcoholism (O'Malley *et al*., 1992; Volpicelli *et al*., 1992), identified before clinical use *via* a large body of preclinical data (see Cowen & Lawrence, 1999). Based on the preclinical and other data, several other neuropeptides may have a significant role in the aetiology of alcoholism (for a recent review see Cowen *et al*., 2004). For example, the peptide corticotropin-releasing factor (CRF) is implicated in stress in general, and also stress-related alcohol use (see Valdez &

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Koob, 2004). We have previously shown that antagonism of CRF_1 receptors by antalarmin attenuates stress-induced acquisition and maintenance of alcohol self-administration (Lodge & Lawrence, 2003). An increasing body of research has also indicated an interaction between neuropeptide Y (NPY) and alcohol consumption, driven by the finding that NPY-deficient mice show increased ethanol consumption, whereas NPY-overexpressing mice show decreased ethanol consumption (Thiele *et al*., 1998). Although the NPY-deficient mice were less sensitive to the sedative/hypnotic effects of ethanol, whereas the NPY-overexpressing mice were more sensitive (Thiele *et al*., 1998), a second strain of NPY-deficient mice on a different genetic background did not demonstrate this decreased sensitivity to the hypnotic effects of ethanol, but did demonstrate greater ethanol consumption than wild-type mice when a 20% solution was offered (Thiele *et al*., 2000). The regulatory effect of NPY on ethanol consumption appears to be mediated *via* the Y1 receptor, as Y1−/− mice show increased ethanol consumption relative to wild-type mice, but normal consumption of sucrose or quinine solutions (Thiele *et al*., 2002a, b). In contrast, Y2−/− mice (the Y2 receptor believed to be an inhibitory autoreceptor on NPY-containing terminals; Naveilhan *et al*., 1999) were shown to have decreased ethanol consumption relative to wild-type controls (Pandey *et al*., 2003). This effect was replicated with the selective Y2 receptor antagonist BIIE0246, which decreased responding for a sweetened ethanol solution (Thorsell *et al*., 2002).

Given the recent findings relating to orexin systems and drug-seeking (Boutrel *et al*., 2005; Harris *et al*., 2005), the ability of orexins to interact with CRF and/or NPY systems may be of relevance to alcoholism. Indeed, CRF-containing fibres make synaptic contact onto hypothalamic orexinergic neurons; exogenously applied CRF depolarises these cells *via* CRF_1 receptors, suggesting the possibility that stress may release CRF onto orexinergic cells that ultimately (*via* the locus coeruleus) drives the increased arousal associated with a stress response (Winsky-Sommerer *et al*., 2004). Moreover, it has been hypothesised that interactions between CRF and orexin may underpin stress-related aspects of drug addiction (Paneda *et al*., 2005; Winsky-Sommerer *et al*., 2005). In a similar manner, orexinergic fibres innervate NPY-containing cells of the arcuate nucleus (Horvath *et al*., 1999a), whereas the hypothermic response to centrally administered orexin A can be prevented with an antibody to NPY (Jaszberenyi *et al*., 2002). Therefore, there is clear evidence that these systems have the capacity to functionally interact; whether these interactions are implicated in alcoholism must await further investigation.

A key problem with alcoholism, as with addiction more generally, is the chronically relapsing nature of the disorder. This behaviour pattern can be modelled in rodents, where numerous studies have demonstrated the ability of drug priming, psychological stress or the re-presentation of cues previously associated with drug availability to reinstate drug-seeking behaviour, even in the absence of subsequent drug-rewards. Moreover, despite differences between these means of reinstating previously extinguished behaviour, there is good general correspondence between animal studies of reinstatement and human experience of relapse (Shaham *et al*., 2003). We have used this paradigm to examine directly the involvement of orexin-containing neurons in cue-induced reinstatement of alcohol-seeking.

Methods

All experiments were performed in accordance with the Prevention of Cruelty to Animals Act, 1986 under the guidelines of the National Health and Medical Research Council Code of Practice for the Care and Use of Animals for Experimental Purposes in Australia. Inbred alcohol-preferring (iP) and -nonpreferring (NP) rats were obtained from the breeding colony at the Howard Florey Institute, University of Melbourne. Parental stock had previously been obtained from Professor T.K. Li (while at Indiana University, Indianapolis, IN, U.S.A.).

Operant responding for alcohol

Alcohol-preferring iP rats ($n=23$) were trained to self-administer ethanol (10% ($v\,v^{-1}$)) under operant conditions using a fixed ratio of 3 (FR3) during 20-min sessions as published previously (Cowen *et al*., 2005a, b; Liang *et al*., 2006). Operant chambers supplied by Med Associates (St Albans, Vermont, CA, U.S.A.) were employed. Each chamber was housed individually in sound-attenuation cubicles, featuring a fan to provide airflow and mask external noise and the chambers were connected to a computer running Med-PC IV software (Med Associates) to record activity. Within the chambers, a house light provided soft illumination during operant sessions. On either side of the operant chambers, a retractable lever (exerted during operant sessions) was centrally placed below a stimulus light and adjacent to a fluid receptacle. Each receptacle was fed by a solenoid-controlled liquid dispenser with a 20 ml reservoir. In addition, availability of ethanol was conditioned by the presence of an olfactory cue (S^+; two drops of vanilla essence, placed on the bedding of the operant chamber directly under the active lever), plus a 1-s light stimulus (CS^+) occurred when the FR3 requirement was obtained.

Training of the rats followed a standard sucrose fade protocol as described previously (Cowen *et al*., 2005a). Ultimately, rats were responding for 10% ethanol solution under an FR3 requirement. For each session, total ethanol and water responses were recorded, and the difference in fluid in the ethanol reservoir between the beginning and end of the session was recorded to ensure correct calibration of the delivery system.

Following acquisition of lever pressing and stable self-administration between sessions, 12 rats were subjected to extinction, during which time there were no cues placed in the operant chamber and there was no programmed response subsequent to task completion. Extinction sessions continued until responding on the 'active' lever was similar to that on the water lever, and stable between trials. Reinstatement was triggered by replacing CS^+, that is, the olfactory cue under the 'active' lever and also reprogramming the software such that the stimulus light was activated (1 s) after every FR3 response, although there was no delivery of ethanol into the receptacle. Rats were injected with either SB-334867 ($20\,mg\,kg^{-1}$, intraperitoneally (i.p.)) or vehicle (3% DMSO, i.p.) 30 min before the commencement of the trial. The dose of SB-334867 and pretreatment timing before behavioural analysis is concordant with other recent studies (Harris *et al*., 2005).

The remaining 11 rats were used to examine the effect of SB-334867 on responding for 10% ethanol under an FR3 schedule

with CS^+. These rats were not subjected to extinction, and were treated with either SB-334867 (20 mg kg^{-1}, i.p.) or vehicle (3% DMSO, i.p.) 30 min before the commencement of the trial. The following week, treatments were reversed.

Neurochemistry

A separate group of 13 age-matched, male iP and six NP rats were used to examine the expression of pre–pro-orexin mRNA in the brain. Six of the iP rats were alcohol naïve, whereas the other seven iP rats were allowed to consume ethanol in a two bottle free-choice paradigm for 70 days. For the first 28 days, rats were given a choice of 5% (v v^{-1}) ethanol or water, whereas for the subsequent 42 days, they had a choice of 10% (v v^{-1}) ethanol or water. The position of drink containers was randomly altered to prevent a side preference. Volumes of fluid consumed and preference for ethanol were calculated daily. At the end of this period, rats were killed by decapitation and their brains removed and frozen over dry ice.

Rat brains were sectioned (14 μm) in a cryostat and slices thaw-mounted onto microscope slides coated in poly-L-lysine. *In situ* hybridisation was performed as described previously (Lawrence *et al*., 1996; Cowen & Lawrence, 2001; Lodge & Lawrence, 2001) using an antisense oligonucleotide to pre–pro-orexin (Ford *et al*., 2005). In brief, oligonucleotide probes (0.3 pmol μl^{-1}) were 3′-end labelled with [α-^{33}P]dATP using the enzyme terminal deoxynucleotidyl transferase at 37°C for 30 min. The labelled oligonucleotide was separated from the reaction products on a sephadex (G25) spin column. Non-specific hybridisation of the probes was examined by the addition of unlabelled oligonucleotide (100-fold molar excess) to the hybridisation mixture. Following hybridisation, sections were washed, dehydrated in ethanol and air-dried before apposition to Kodak X-ray film in the presence of standard microscales. Densitometric analysis of each brain section was performed against known microscales (^{14}C, American Radiolabeled Chemicals Inc., St Louis, U.S.A.) using the SCION imaging system (PC version of NIH image) with reference to a stereotaxic atlas (Paxinos & Watson, 1986).

Data analysis

All data are represented as the mean ± s.e.m. unless otherwise specified. Statistical analysis was performed using SigmaStat software. Operant responding data were analysed by one-way ANOVA with Student–Newman–Keuls *post hoc* tests. Differences in mRNA expression between groups were performed on raw data (d.p.m. mm^{-2} or area of expression in mm^2) using one-way ANOVA with Student–Newman–Keuls *post hoc* tests.

Results

Cue-induced reinstatement of alcohol-seeking

Before extinction, alcohol-preferring iP rats responded stably and preferentially for 10% ethanol (145 ± 12 responses) compared with water responding (11 ± 1 responses), corresponding to an average ethanol intake of 0.82 ± 0.07 g kg^{-1} per session. Over 15 sessions, ethanol responding was extinguished (no availability of ethanol or water and no presentation of ethanol-associated cues; ethanol responses: 11 ± 6; water responses: 9 ± 7; Figure 1). Re-presentation of the cues previously associated with ethanol availability caused a robust and significant increase in responding on the active (ethanol) lever in vehicle (3% DMSO)-treated rats that was completely prevented by SB-334867 (20 mg kg^{-1} i.p., 30 min prior: $F_{(2,21)} = 25.415$, $P < 0.005$; Figure 1). Responding on the inactive lever remained unaffected by 20 mg kg^{-1} SB-334867 ($F_{(2,21)} = 2.803$, $P = 0.083$; Figure 1).

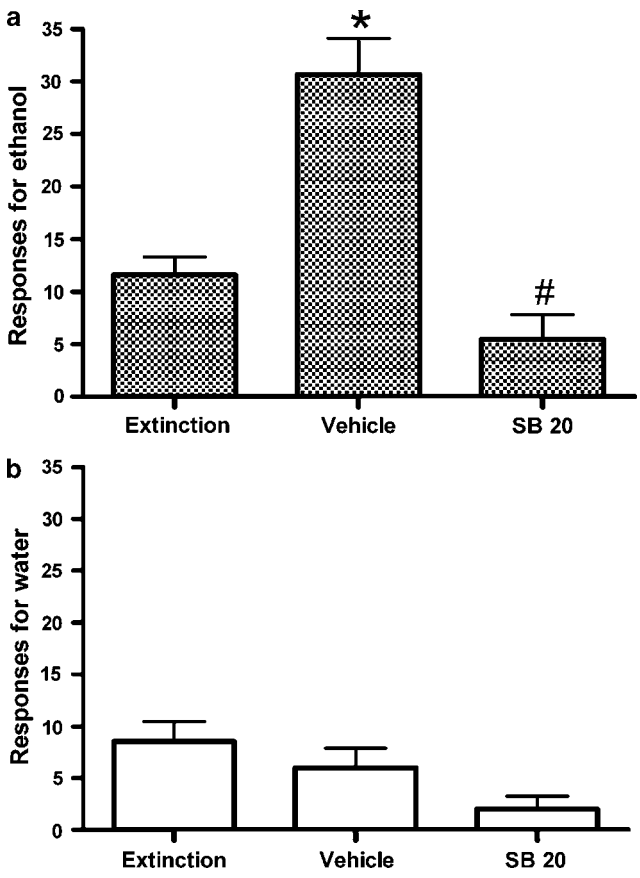

Figure 1 Extinction and cue-induced reinstatement of alcohol-seeking in iP rats ($n = 12$). SB-334867 (20 mg kg^{-1}, i.p.) specifically abolishes responding for alcohol (a), without affecting responding for water (b) upon reinstatement. Statistical analysis was performed using one-way ANOVA with Student–Newman–Keuls *post hoc* tests. Data are mean ± s.e.m. $^*P < 0.05$ compared to extinction; $^{\#}P < 0.05$ compared to vehicle.

Operant responding for alcohol under an FR regimen

A separate cohort of 11 male iP rats were used to examine the effect of SB-334867 (20 mg kg^{-1} i.p., 30 min prior) on operant responding for ethanol and water under an FR regimen (Figure 2). SB-334867 (20 mg kg^{-1}) caused a significant decrease in ethanol responding ($F_{(2,20)} = 11.762$, $P < 0.001$; Figure 2a) and the number of times the ethanol receptacle was accessed ($F_{(2,20)} = 7.885$, $P = 0.003$; Figure 2b) with no significant effect on water responding ($F_{(2,20)} = 1.667$, $P = 0.214$; Figure 2c) or the number of times the water receptacle was accessed ($F_{(2,20)} = 0.186$, $P = 0.832$; Figure 2d).

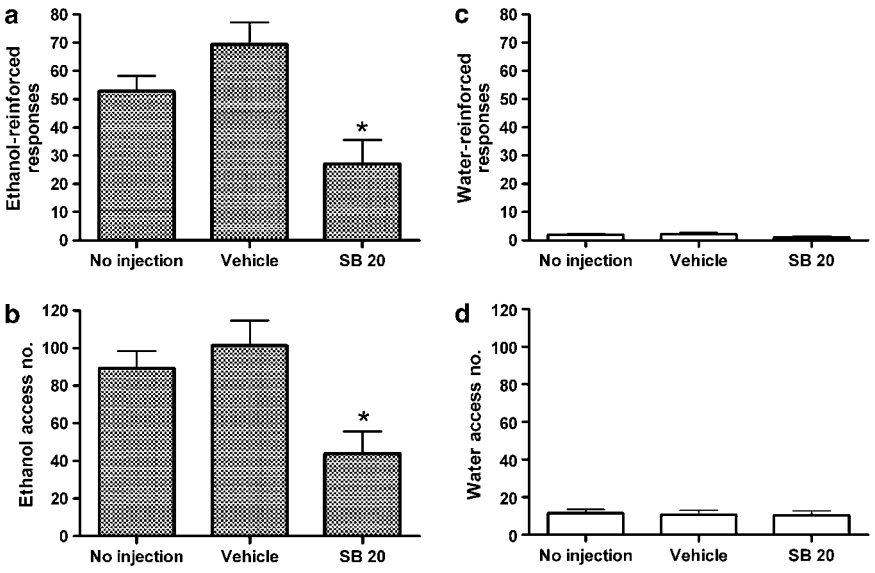

Figure 2 Operant responding for 10% ethanol and water in iP rats ($n=11$) under an FR3 schedule. SB-334867 (20 mg kg^{-1}, i.p.) reduces operant responding for ethanol (a) and consumption of ethanol (c) without impacting upon responding for water (b) or consumption of water (d). Data are mean ± s.e.m. *$P<0.05$ compared to vehicle, one-way ANOVA with Student–Newman–Keuls *post hoc* tests.

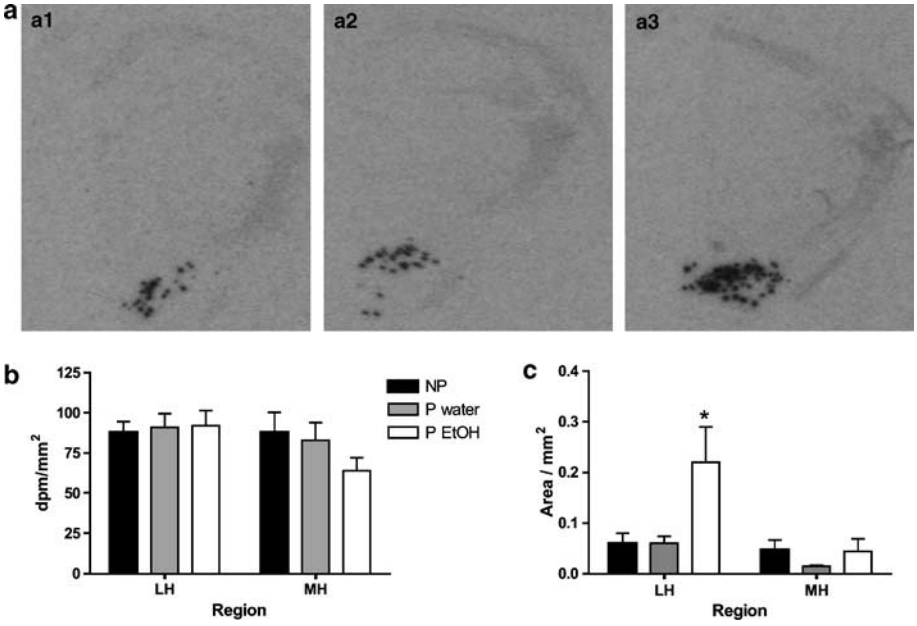

Figure 3 Expression of the mRNA encoding pre–pro-orexin in iP and NP rat hypothalamus (a). a1 is a representative section from an NP rat, a2 from an alcohol-naïve iP rat and a3 from an iP rat following chronic alcohol consumption. Chronic consumption of ethanol had no impact upon the density of mRNA expression (b), but did increase the area of dense pre–pro-orexin mRNA expression within the lateral hypothalamus (c). Data are mean ± s.e.m. ($n=6$–7 rats/group). *$P<0.05$, one-way ANOVA with Student–Newman–Keuls *post hoc* tests.

Neurochemistry

Over the 10-week-period, under a two bottle free-choice paradigm, iP rats consumed ~5 g kg^{-1} ethanol per day with ~80% preference. Orexin mRNA was densely expressed around the medial and lateral hypothalamus (Figure 3a). There was no significant difference between NP, alcohol-naïve iP or iP rats following chronic ethanol consumption in terms of density of mRNA expression in the lateral hypothalamus ($F_{(2,17)}=0.090$, $P=0.914$). In contrast however, the area in the lateral hypothalamus in which orexin mRNA was expressed was markedly increased in alcohol-preferring iP rats that had access to ethanol ($F_{(2,17)}=5.145$, $P=0.018$) compared with both alcohol-naïve iP rats and alcohol -nonpreferring NP rats (Figure 3b and c). This effect was not observed in the medial hypothalamus.

Discussion

These data provide the first direct evidence that orexin$_1$ receptors are implicated in the expression of cue-induced reinstatement of alcohol-seeking behaviour. A recent article (Mileykovskiy *et al*., 2005) suggested that orexin-containing neurons are strongly activated by foraging, which has supported the finding that orexins appear to be involved in drug-seeking behaviour (Harris *et al*., 2005). Consequently, it has been suggested that the potential of a reward may activate the orexin neurons of the lateral hypothalamus, but if exploration is not rewarded then the orexin neurons may not be activated (Scammell & Saper, 2005). In the context of operant studies, the action of lever pressing may equate to foraging in terms of seeking out and anticipating a reward. The present data clearly demonstrate that prior antagonism of orexin$_1$ receptors by SB-334867 completely abolishes responding on the active lever during a reinstatement session. This would suggest that integration of the cues previously associated with alcohol availability is sufficient and adequate to evoke the release of endogenous orexin to drive this behaviour. Most importantly, the fact that this behaviour persists (in vehicle-treated rats) in the absence of any subsequent alcohol delivery suggests that orexin is released in anticipation of drug availability, and that orexin continues to be released to maintain the alcohol-seeking, at least for the duration of the operant session.

The question therefore arises as to how antagonism of orexin$_1$ receptors totally prevents a cue-induced reinstatement of alcohol-seeking (as well as self-administration of alcohol). Recently, SB-334867 has been demonstrated to antagonise effectively foot-shock stress-induced reinstatement of cocaine-seeking behaviour (Boutrel *et al*., 2005). This latter study also demonstrated that the ability of orexin agonists to stimulate a reinstatement of cocaine-seeking was sensitive to blockade by manipulation of the noradrenergic- and corticotropin-releasing factor systems, suggesting that generalised satiety signals are of little importance in this specific context, but rather activation of stress pathways predominate. Given the role of CRF in aspects of alcohol consumption (see Introduction), on the surface therefore, one could possibly surmise that SB-334867 is somehow interfering with alcohol-related stress systems, thereby reducing self-administration and reinstatement. Although this explanation may be appealing at one level, there are a number of problems with this explanation. There is clear evidence that extinguished drug-seeking can be reinstated by drug priming, stressors and/or the reintroduction of specific drug-related cues (see Shaham *et al*., 2003). Most importantly, the brain systems and chemicals involved in these different means of precipitating reinstatement differ. Crucially, in the context of reinstatement to alcohol-seeking in rats, CRF_1 receptor antagonists can prevent a stress-induced reinstatement, but not a cue-induced reinstatement (Liu & Weiss, 2002). The present data reflect the latter case, reinstatement is precipitated by re-exposure to an olfactory cue previously paired with the availability of alcohol. Therefore, it is unlikely that the ability of SB-334867 to prevent a cue-induced reinstatement relates to interactions with CRF stress systems. Accordingly, it would appear that the orexin system is possibly a common component in the networks that integrate both stressors and conditioned cues with respect to drug-seeking.

Another possibility is that antagonism of orexin$_1$ receptors results in an increased release of NPY that ultimately attenuates the cue-induced reinstatement of alcohol-seeking. While exogenously applied NPY can reduce self-administration of alcohol following an enforced abstinence (Gilpin *et al*., 2003; 2005), at present there are no data relating to the ability of NPY to prevent cue-induced reinstatement of alcohol-seeking.

Perhaps a more probable explanation, based on current knowledge, is that SB-334867 is antagonising the ability of endogenous orexin (released upon integration of the olfactory cue) to activate directly pathways involved in drug-seeking. In this scenario, the ventral tegmental area is a candidate structure (Fadel & Deutch, 2002; Baldo *et al*., 2003; Korotkova *et al*., 2003). Recent evidence suggests that the dopamine-containing cells of the VTA are a distinct target for the ability of orexin to regulate dopamine release and behavioural responses to opiates (Narita *et al*., 2006) and cocaine (Borgland *et al*., 2006). Moreover, the conditioned rewarding effect of ethanol is expressed through a VTA-dependent mechanism (Bechtholt & Cunningham, 2005). This factor is possibly more important than sensitisation in terms of the ability of conditioned (olfactory) cues to reinstate alcohol-seeking. While it has been suggested that intra-VTA injections of orexins increase the release of dopamine into the prefrontal cortex but not the nucleus accumbens (Vittoz & Berridge, 2006), this does not preclude the VTA as a site from where SB-334867 can prevent reinstatement of alcohol-seeking. Thus, the prefrontal cortex is likely involved in the integration of the salience of the cue, and additionally, VTA cells also project to the amygdala, which is another potential substrate.

The present study used a dose of SB-334867 ($20\,mg\,kg^{-1}$, i.p.) previously used in other studies (Rodgers *et al*., 2001), which has been demonstrated not to reduce locomotor activity, grooming or eating. Moreover, preliminary studies with a higher dose of SB-3334867 ($30\,mg\,kg^{-1}$, i.p., data not shown), as used in some studies (e.g. Harris *et al*., 2005), resulted in nonspecific behavioural effects (unpublished observation); these were not observed when we titrated the dose to $20\,mg\,kg^{-1}$ whereupon a specific effect on alcohol responding was noted. Indeed, SB-334867 ($30\,mg\,kg^{-1}$, i.p.) has been demonstrated to reduce feeding, locomotion and increase resting (Rodgers *et al*., 2001). Collectively, these findings would suggest that SB-334867 should be used at doses of $20\,mg\,kg^{-1}$ or lower to prevent potentially confounding behaviours from interfering with data interpretation.

It is noteworthy to consider the possibility that SB-334867 may simply act to signal a state of satiety and thereby reduce responding for alcohol. While behavioural similarities have been observed between SB-334867 and cholecystokinin (CCK) in certain paradigms (Ishii *et al*., 2005), the ability of endogenous CCK or exogenous CCK receptor agonists to reduce alcohol consumption is highly transient, with multiple investigators reporting an effect that lasts for no more than 30 min (Kulkosky *et al*., 1993; Geary *et al*., 2004). In our paradigm, the time frame of our drug pretreatment plus operant session would be incompatible with a simple CCK-mediated satiation consequent to antagonism of orexin systems. Moreover, SB-334867 ($20\,mg\,kg^{-1}$) had no effect on water responding, either under reinstatement or an FR3 schedule of appetitive responding. Collectively therefore, these data suggest that acute antagonism of orexin$_1$ receptors can

regulate alcohol-seeking behaviour under operant conditions. This conclusion is in accordance with the observation that orexin-containing cells are most dramatically activated during exploratory behaviour (Mileykovskiy *et al*., 2005), as would be the case under an operant paradigm.

To examine whether or not differences in orexin mRNA expression may contribute to the alcohol-preferring phenotype of iP rats, we next performed *in situ* hybridisation to compare between alcohol-naïve iP and -NP rats, and also iP rats following chronic ethanol consumption. There were no differences in the density of pre–pro-orexin mRNA expression between alcohol naïve NP and iP rats. This would suggest that, in the absence of any alteration to post-translational processing, the basal orexin systems are similar between these rat strains and seemingly, therefore, not implicated in contributing to the alcohol-preferring nature of iP rats. Interestingly, chronic consumption of ethanol for 10 weeks in iP rats also had no significant impact upon the density of pre–pro-orexin mRNA expression in the hypothalamus; however, long-term ethanol consumption resulted in an increase in the area of mRNA expression compared to NP and alcohol-naïve iP rats. Notably, this effect was only apparent in the lateral hypothalamus and not in the more medial regions. These data therefore suggest that orexin neurons in the medial hypothalamus are somewhat insensitive to regulation by chronic ethanol consumption, at least under a continual access paradigm. In contrast, chronic volitional consumption of alcohol by iP rats apparently increases the number of neurons that densely express orexin mRNA within the lateral hypothalamus. It would therefore appear that while chronic ethanol consumption does not apparently activate cells that express high basal levels of orexin, it somehow signals surrounding cells (that under basal conditions express low/undetectable levels of orexin) to produce increased amounts of orexin. It should also be noted that, given the behavioural data, it may be that enforced withdrawal from chronic alcohol consumption may be a stronger stimulus to activate orexin-containing cells. Clarification of these issues awaits further experimentation. Whatever the explanation, the present data do not support a clear role for differential expression of pre–pro-orexin with alcohol preference *per se*.

Therefore, we have provided two independent, yet complementary, measures relating orexin-containing neurons to alcohol consumption and abuse. Firstly, in an acute behavioural paradigm, antagonism of orexin$_1$ receptors reduces alcohol responding under an FR paradigm, and also abolishes cue-induced reinstatement of alcohol-seeking behaviour in iP rats. Secondly, while the expression of the mRNA encoding pre–pro-orexin is similar between naïve iP and iNP rats, chronic consumption of ethanol appears to recruit orexin-expressing neurons in the lateral hypothalamus. Clearly, these two experiments are not designed to be compared between each other, rather they represent different approaches to a central theme. These data are in accordance with recent findings that indicated a strong correlation between activation of lateral hypothalamic orexin-containing cells and conditioned place preference for morphine, cocaine and food (Harris *et al*., 2005). Also consonant with this hypothesis is a recent suggestion of an involvement of orexin neurons in a hypothalamic–thalamic–striatal network implicated in the regulation of behavioural state, feeding and reward (Kelley *et al*., 2005).

In conclusion, these data demonstrate that cues associated with the availability of alcohol are robust activators of orexin systems, and furthermore, orexin acting upon orexin$_1$ receptors is implicated in the process of cue-induced reinstatement of alcohol-seeking. These findings provide a definitive rationale for the identification of central orexin systems as valid targets for the pharmacotherapy of alcohol, and possibly other substance, abuse.

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