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Research report

# Orexin/hypocretin-1 receptor antagonism reduces ethanol self-administration and reinstatement selectively in highly-motivated rats

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ABSTRACT

The orexin/hypocretin (ORX) system regulates motivation for natural rewards and drugs of abuse such as alcohol. ORX receptor antagonists, most commonly OX1R antagonists including SB-334867 (SB), decrease alcohol drinking, self-administration and reinstatement in both genetically-bred alcohol-preferring and outbred strains of rats. Importantly, levels of alcohol seeking and drinking in outbred rats are variable, as they are in humans. We have shown that OX1R antagonism selectively decreases homecage alcohol drinking in high-, but not low-alcohol-preferring rats. It is unknown, however, whether this effect is selective to homecage drinking or whether it also applies to alcohol seeking paradigms such as self-administration and reinstatement following extinction, in which motivation is high in the absence of alcohol. Here we trained Sprague Dawley rats to self-administer 20% ethanol paired with a light-tone cue on an FR3 regimen. Rats were then extinguished and subjected to cue-induced reinstatement. Rats were segregated into high- and low-ethanol-responding groups (HR and LR) based on self-administration levels. During self-administration and cue-induced reinstatement, rats were given SB or vehicle prior to ethanol seeking. In both conditions, OX1R antagonism decreased responding selectively in HR, but not LR rats. There were no non-specific effects of SB treatment on arousal or general behavior. These data indicate that ORX signaling at the OX1R receptor specifically regulates high levels of motivation for alcohol, even in the absence of direct alcohol reinforcement. This implicates the ORX system in the pathological motivation underlying alcohol abuse and alcoholism and demonstrates that the OX1R may be an important target for treating alcohol abuse.

## 1. Introduction

Orexin (ORX, also known as hypocretin, HCRT) neurons are located in a limited region of the dorsal hypothalamus consisting of the lateral, perifornical, and dorsomedial hypothalamic areas (de Lecea et al., 1998; Peyron et al., 1998; Sakurai et al., 1998). These neurons project throughout the brain (Sakurai et al., 2005; Yoshida et al., 2006), and are thought to regulate a wide range of functions including arousal and reward motivation, among a number of others (Aston-Jones et al., 2010; Li et al., 2014; Mahler et al., 2014; Sakurai, 2007, 2014). ORX neurons produce two peptides, ORX-A and ORX-B (or HCRT-1 and HCRT-2) (de Lecea et al., 1998; Sakurai et al., 1998). These peptides differentially bind to two ORX receptors – the ORX-1 receptor (OX1R; HCRT1R) and the ORX-2 receptor (OX2R; HCRT2R) (de Lecea et al., 1998; Sakurai et al., 1998). The OX1R exhibits stronger selectivity for ORX-A, whereas the OX2R exhibits approximately equal selectivity for ORX-A and ORX-B (Sakurai et al., 1998). These receptors are distributed differentially across the brain (Marcus et al., 2001), and a number of studies have indicated that they likely play different roles in physiological function and behavior (Mahler et al., 2012, 2014). Although not absolute, it has been hypothesized that whereas the OX2R is important in regulating the arousal-related functions associated with the ORX system, the OX1R plays a more important role in controlling the motivational functions of the ORX system (Mahler et al., 2012, 2014).

Of particular importance, the OX1R has been widely associated

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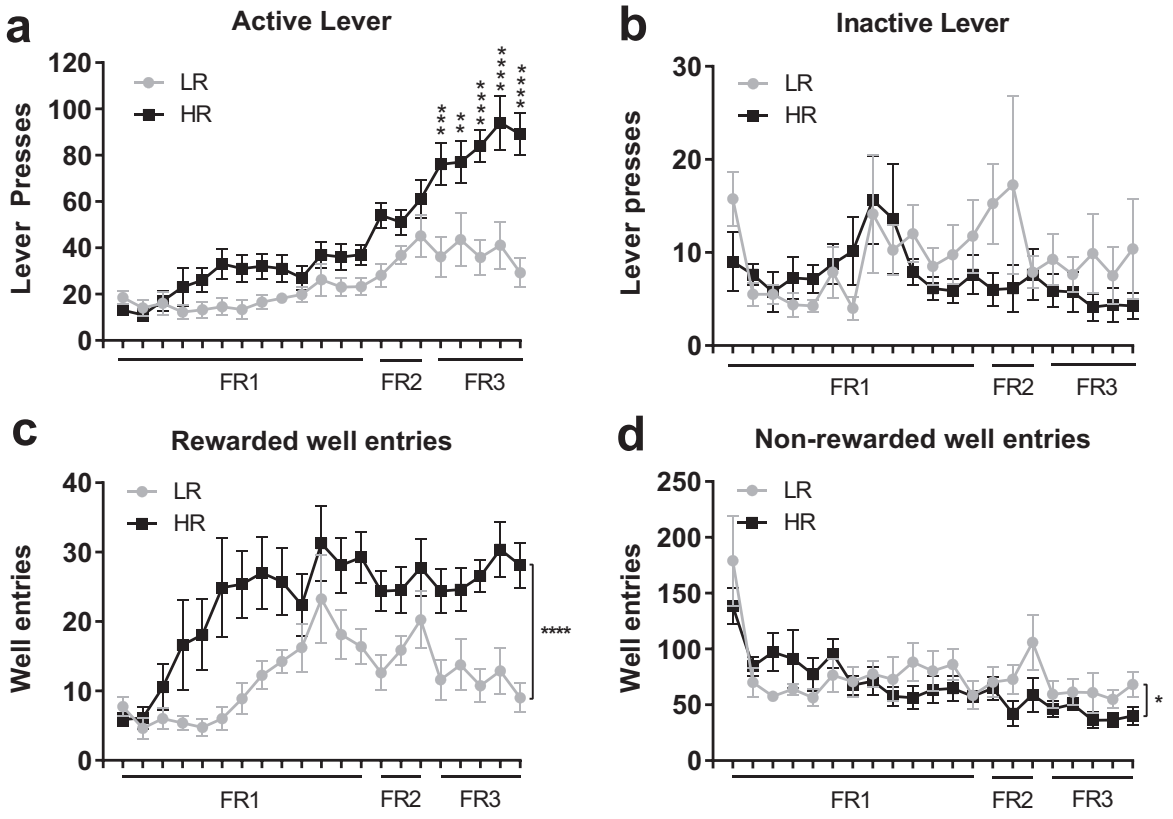


**Fig. 1.** *Comparison of alcohol self-administration behavior in HR vs LR rats.* (A) HR animals showed significantly greater active lever responses during FR3 sessions. (B) There were no differences between HR and LR animals in terms of inactive lever responses at any stage of self-administration training. (C) Similar to active lever responses, HR animals exhibited a significantly greater number of rewarded well entries across all self-administration sessions. (D) HR and LR animals showed similar levels of non-rewarded well entries during self-administration training. *p < 0.05; ** p < 0.01; *** p < 0.001; **** p < 0.0001.

with motivation for drugs of abuse, including alcohol (Brown et al., 2013a; Dayas et al., 2008; Jupp et al., 2011; Lawrence et al., 2006; Mahler et al., 2012; Martin-Fardon and Weiss, 2014; Moorman and Aston-Jones, 2009; Moorman et al., 2016). The OX1R-selective antagonist SB-334867 (SB) decreases seeking of multiple drugs of abuse (Bentzley and Aston-Jones, 2015; James et al., 2012; Mahler et al., 2012; Plaza-Zabala et al., 2012; Porter-Stransky et al., 2015). There is a particularly (though not exclusively: Anderson et al., 2014; Barson et al., 2015; Brown et al., 2013b; Shoblock et al., 2011) strong relationship between the OX1R vs OX2R and alcohol seeking. SB decreased cue-induced reinstatement of alcohol seeking in alcohol-preferring rats (Jupp et al., 2011; Lawrence et al., 2006), decreased stress-induced reinstatement of alcohol seeking in Long-Evans rats (Richards et al., 2008), and decreased reinstatement of alcohol-seeking elicited by discriminative stimuli in Wistar rats (Martin-Fardon and Weiss, 2014). SB also decreased relapse to alcohol seeking/drinking after homecage deprivation in female alcohol-preferring rats, but only when alcohol was available (Dhaher et al., 2010). These effects are mediated, at least in part, by OXR1 signaling in the ventral tegmental area and prelimbic cortex, as SB infused into these regions independently decreased cue-induced ethanol seeking (Brown et al., 2016).

OX1R antagonism also decreases alcohol drinking. SB treatment decreased alcohol drinking in alcohol-preferring rats (Anderson et al., 2014) as well as in Sprague Dawley rats (Moorman and Aston-Jones, 2009) and C57BL/6J mice (Anderson et al., 2014; Olney et al., 2015). Antagonism of OX2R also has an effect on ethanol seeking in the presence of ethanol (Brown et al., 2013b; Shoblock et al., 2011), indicating potential differential contributions of ORX signaling at each receptor, possibly due to differential receptor distribution (Cluderay et al., 2002; Hervieu et al., 2001; Marcus et al., 2001; Trivedi et al., 1998).

We previously demonstrated that OX1R antagonism decreased two-bottle choice preference selectively in high-alcohol-preferring, and not in low-alcohol-preferring Sprague Dawley rats (Moorman and Aston-Jones, 2009). We also showed that the selective OX1R antagonist GSK1059865 decreased alcohol drinking preferentially in mice that had increased ethanol drinking following chronic intermittent access to ethanol (Lopez et al., 2016). These results align with findings in ethanol-preferring rats (Jupp et al., 2011; Lawrence et al., 2006), and extend those results to show that the effect of OX1R antagonism on alcohol drinking is maximally efficacious for individuals with high motivation to drink alcohol, compared to those with low motivation. These findings are also consistent with Fos activation of ORX neurons, in which strength of such activation typically correlates with alcohol seeking (Hamlin et al., 2007; Moorman et al., 2016). Taken together, these results indicate that OX1R treatment may be particularly important in individuals prone to alcohol abuse or addiction. However, the selective effect of OX1R antagonism has not yet been demonstrated on alcohol seeking in operant self-administration contexts or reinstatement paradigms, which model human alcohol seeking and relapse (Shaham et al., 2003). In the present study, we investigated the effects of OX1R antagonism on alcohol self-administration and cue-induced reinstatement in Sprague Dawley rats that exhibited high or low levels of alcohol seeking behavior. Intriguingly, we found that rats segregated into low- vs. high-responders as response demands increased (at the transition to FR3 seeking) and that this segregation was consistent over time. When treated with SB, high-responding animals exhibited decreases in ethanol self-administration and cue-induced reinstatement whereas low-responding animals were not affected. These results demonstrate a strong connection between alcohol seeking and ORX system, particularly the OX1R, and indicate that this system may be fundamentally involved in alcohol use disorders.