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Lateral hypothalamic orexin/hypocretin neurons: A role in reward-seeking and addiction

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Abstract

Orexins (synonymous with hypocretins) are recently discovered neuropeptides made exclusively in hypothalamus. Behavioral, anatomical and neurophysiological studies show that a subset of these cells, specifically those in lateral hypothalamus (LH), are involved in reward processing and addictive behaviors. Fos expression in LH orexin neurons varied in proportion to conditioned place preference (CPP) for morphine, cocaine or food. This relationship occurred both in drug naïve rats and in animals during protracted morphine withdrawal, when drug preference was elevated but food preference was decreased. Inputs to the LH orexin cell field from lateral septum and bed nucleus of the stria terminalis were Fos activated during cocaine CPP in proportion to the preference expressed in each animal. This implies that these inputs may be involved in driving the conditioned responses in LH orexin neurons. Related studies showed that LH orexin neurons that project to ventral tegmental area (VTA) had greater Fos induction in association with elevated morphine preference during protracted withdrawal than non-VTA-projecting orexin neurons, indicating that the VTA is an important site of action for orexin's role in reward processing. In addition, stimulation of LH orexin neurons, or microinjection of orexin into VTA, reinstated an extinguished morphine preference. In self-administration studies, the orexin 1 receptor antagonist SB-334867 (SB) blocked cocaine-seeking induced by discrete or contextual cues previously associated with cocaine, but not by a priming injection of cocaine. There was no effect of SB on cocaine self-administration itself, indicating that it did not interfere with the drug's reinforcing properties. Neurophysiological studies revealed that locally applied orexin often augmented responses of VTA dopamine (DA) neurons to activation of the medial prefrontal cortex (mPFC), consistent with the view that orexin facilitates activation of VTA DA neurons by stimulus-reward associations. This LH-to-VTA orexin pathway was found to be necessary for learning a morphine place preference. These findings are consistent with results showing that orexin facilitates glutamate-mediated responses, and is necessary for glutamate-dependent long-term potentiation in VTA DA neurons. We surmise from these studies that LH orexin neurons play an important role in reward processing and addiction, and that LH orexin cells are an important input to VTA for behavioral effects associated with reward-paired stimuli.

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Introduction

The neuropeptides orexin A and orexin B (synonymous with hypocretin 1 and hypocretin 2) are produced from a prepro-orexin molecule made solely in hypothalamic neurons. Since their discoveries by de Lecea et al. and Sakurai et al. in 1998 (de Lecea et al., 1998; Sakurai et al., 1998), considerable work has characterized this neurotransmitter system. Sakurai et al. (Sakurai et al., 1998) characterized two receptors for the orexin system, termed OxR1 and OxR2 (also denoted HcrtR1 and HcrtR2). OxR1 binds orexin A with 30 nM affinity but has much lower affinity for orexin B, whereas OxR2 binds both orexin peptides with similar high affinity. Further, OxR1 is coupled exclusively to a G_q subclass of G proteins, and OxR2 is coupled to both G_q and $G_{i/o}$ proteins (Zhu et al., 2003). The orexin neurons give rise to a highly divergent system of fiber projections that spans the entire neuraxis, including innervation in the cerebral cortex, hippocampus, thalamus, midbrain, and spinal cord (Peyron et al., 1998; Sutcliffe and de Lecea, 2002). Likewise, the two orexin receptors are widely distributed in the CNS, but are regionally selective and largely non-overlapping (Kilduff and de Lecea, 2001; Lu et al., 2000; Marcus et al., 2001; Trivedi et al., 1998).

Great interest was focused on this system shortly after its discovery, when two groups virtually simultaneously reported that dysfunction in the orexin system is strongly associated with narcoleptic symptoms in animals (Chemelli et al., 1999; Lin et al., 1999). Subsequent work in humans verified that narcoleptic patients (particularly those with cataplexy) have little orexin in their CSF, and lack most or all orexin neurons (Nishino et al., 2000; Nishino, 2007). With these compelling findings, the prevailing view of orexin function focused on arousal and maintenance of the waking state. Supporting this view were findings that major targets of orexin projections are classic brain arousal nuclei such as the locus coeruleus (Peyron et al., 1998; Sutcliffe and de Lecea, 2002), and that orexin application typically strongly activates these cells (Brown et al., 2001; Eriksson et al., 2001; Horvath et al., 1999; Ivanov and Aston-Jones, 2000; Korotkova et al., 2003).

However, a potential role for orexins in reward processing was evident from one of the first publications of their discovery. Sakurai and colleagues reported that administration of orexin A or orexin B into the lateral ventricle produced feeding in rats, which prompted them to name the new peptides “orexins”, meaning appetite (Sakurai et al., 1998). The first report of a possible role for orexins in effects of addictive drugs appeared in 2003, and showed that orexin neurons play a role in opiate withdrawal (Georgescu et al., 2003). Subsequent studies examined a possible role for this novel neuropeptide system in reward processing and drug abuse. As reviewed below, orexin appears to play a prominent role in conditioned responses to stimuli associated with food and drug rewards. This reward-associated function of the orexin system may be separate from its role in maintenance of the waking state, and mediated by a separate population of (laterally located) orexin neurons.

Orexins and drug-stimulus associations

Orexin neurons are activated by reward-associated stimuli

Orexin neurons in lateral hypothalamus (LH) play an active role in reward processing and drug abuse. Mice with a genetic deletion of orexin completely lack conditioned place preference (CPP) for morphine (Narita et al., 2006). Further, there is a strong association between Fos activation in orexin neurons and the expression of CPP for drug or natural rewards (Harris et al., 2005). Rats conditioned with morphine, cocaine or food in a CPP paradigm exhibited substantially increased Fos staining in LH orexin neurons on the drug- and food-free CPP test session (Fig. 1, Table 1). Notably, this Fos induction in LH orexin neurons was in close proportion to the degree of preference that animals exhibited on the CPP test day ($r = 0.72$ to 0.90, $p<0.01$). Moreover, this behavioral correspondence with Fos induction was selective for

orexin neurons in the lateral part of the orexin cell field (in LH), and was not found for orexin neurons outside LH (i.e., perifornical, PeF, or dorsomedial hypothalamus, DMH), nor for non-orexin neurons within LH (Table 1). Note also that LH orexin neurons have a lower baseline (constituitive) level of Fos expression, and show a larger increase in Fos with the CPP test, than PeF or DMH orexin neurons. Self-administration studies found Fos activation in orexin neurons after exposure to ethanol-associated stimuli (Dayas et al., 2008), but also showed Fos activation only in non-orexin LH neurons in response to cocaine-associated contexts (Hamlin et al., 2008), indicating that Fos expression in orexin neurons might vary depending on the drug or self-administration paradigm.

Systemic administration of the OxR1 antagonist, SB-334867 (SB) before the CPP test session significantly attenuated expression of a morphine preference (Harris et al., 2005), indicating that activation of LH orexin neurons plays a role in driving the associated preference. To determine whether conditioned activation of orexin neurons is also involved in driving reinstatement of extinguished drug-seeking, rats were conditioned for morphine CPP, and then underwent extinction of morphine preference by repeatedly exposing them to the CPP environment without drug reward. After achieving extinction of preference (which typically required 1–3 weeks), the Y4 receptor agonist rat pancreatic polypeptide (rPP) was microinjected into LH to stimulate orexin neurons. Campbell and colleagues (Campbell et al., 2003) showed previously that rPP preferentially stimulates Fos induction in orexin neurons when injected into LH. rPP microinjected into LH produced a robust reinstatement of preference for the previous (extinguished) morphine-paired side (Fig. 2). This effect was specific to LH, as injections dorsal to LH, or medial to LH among non-LH orexin neurons, were not effective in reinstating preference (Harris et al., 2005). This reinstatement with LH rPP was also specific for orexin neurotransmission, as it was completely blocked by systemic pretreatment with SB. Intracerebroventricular administration of orexin A was also found to reinstate an extinguished cocaine-seeking response in the self-administration paradigm, further indicating that orexin can drive drug-seeking and relapse (Boutrel et al., 2005b).

Orexin is necessary for conditioned responding but not cocaine reinforcement

The relationship between the orexin system and stimulus-drug associations has been further demonstrated using the reinstatement model of relapse in the self-administration paradigm (Smith et al., 2009a; Smith et al., 2009b). For these studies, rats self-administered intravenous cocaine (0.2 mg/infusion) during daily 2-hour sessions, followed by extinction training in the operant chamber or abstinence in the home cage. Reinstatement of extinguished cocaine-seeking was elicited by one of the following: presentation of discrete cues previously paired with cocaine infusions, cocaine prime (10 mg/kg, i.p.), or re-exposure to the original self-administration context. In abstinent (non-extinguished) animals, relapse was elicited by re-introduction to the self-administration environment.

Systemic administration of 20–30 mg/kg SB (i.p.) significantly attenuated cue-induced reinstatement of extinguished cocaine-seeking, as compared to reinstatement following vehicle injection in the same animals (Fig. 3) (Smith et al., 2009a). Importantly, SB had no effect on lever responding during a late extinction trial with no cues, indicating that the reinstatement effect was not due to general effects of the antagonist on locomotion or arousal. These results correspond with previous findings that SB reduced cue-induced reinstatement of extinguished ethanol-seeking (Lawrence et al., 2006). In contrast to the effectiveness in blocking cue-induced reinstatement of cocaine-seeking, 10 or 30 mg/kg SB did not attenuate reinstatement of responding induced by a priming injection of cocaine (Smith et al., 2007). Animals similarly reinstated lever-pressing after a cocaine prime whether pretreated with vehicle or the antagonist. These results suggest that antagonism of OxR1 attenuates conditioned cocaine-seeking, but does not block the priming properties of cocaine.

Further evidence that signaling at OxR1 is involved in conditioned cocaine-seeking comes from recent studies examining context-induced reinstatement. In addition to relapse driven by exposure to discrete cues, relapse to drug-seeking in both human addicts and animal models can be triggered by re-exposure to contextual cues associated with the environment in which drug self-administration occurred (Crombag et al., 2002; Fuchs et al., 2005). As discussed above, orexin neurons show increased activation, as revealed by Fos expression, upon exposure to contextual stimuli associated with several drugs of abuse (Dayas et al., 2008; Harris and Aston-Jones, 2006; Harris et al., 2005). Therefore, the orexin system may be involved in context-elicited cocaine-seeking. In the ABA design, also known as a renewal procedure, animals learn to self-administer drug in the presence of a set of contextual cues (context A) followed by extinction of drug-responding in a different context (context B). Re-exposure to the original self-administration context results in reinstatement of the drug-seeking response (Bouton and Bolles, 1979). In this ABA design, systemic pretreatment with 10–30 mg/kg SB prior to return to the original context significantly attenuated reinstatement of cocaine-seeking as compared to a vehicle-treated group (Fig. 4) (Smith et al., 2009b; Tahsili-Fahadan et al., 2009).

We also found that the orexin system is involved in contextual-driven cocaine-seeking in animals that have undergone abstinence from chronic self-administration without explicit extinction training (Smith et al., 2009b; Tahsili-Fahadan et al., 2009). Following 2 weeks of abstinence in the home cage, 10–30 mg/kg SB significantly attenuated cocaine-seeking upon re-exposure to the self-administration context (Fig. 3). Cocaine-seeking following extinction versus abstinence relies on distinct, but partially overlapping, neural circuitry (Fuchs et al., 2006b); the fact that orexin transmission is needed for both of these reinstatement mechanisms indicates that orexin might be playing a general role in conditioned cocaine-seeking, and acting in brain structures that are common to both types of drug-seeking. These findings, together with above results, reveal that signaling at Ox1R plays a general role in relapse to cocaine-seeking whether elicited by discrete or contextual cues, and is most likely interacting with neurocircuitry common to different relapse-inducing factors.

The role of the orexin system in drug-taking and -seeking depends upon the drug being tested and the reinstating stimulus being used. SB significantly attenuated stress- or yohimbine-induced reinstatement of ethanol- or sucrose-seeking (Boutrel et al., 2005a; Richards et al., 2008). However, administration of 30 mg/kg SB during established cocaine self-administration produced no significant effects on the number of lever presses or drug infusions as compared to self-administration the day before and after, when no pretreatment was given (Smith et al., 2009a). SB also had no effect on self-administration of sucrose, but significantly reduced self-administration of ethanol, nicotine, and high-fat food (Hollander et al., 2008; Lawrence et al., 2006; Nair et al., 2008; Richards et al., 2008). In outbred Sprague-Dawley rats, SB also decreased ethanol intake in those individuals that showed high preference for ethanol; little effect was observed in rats that exhibited low ethanol preference before SB (Moorman and Aston-Jones, 2009). These results indicate that orexin may be necessary for the reinforcing properties of some rewards, and in some individuals, but not others. Possible mechanisms for these differences are discussed in the Discussion. However, in all cases, orexin was found to be critical for drug-seeking elicited by external triggers, such as cues or context associated with drug.

Orexin involvement in learning drug-stimulus associations

The findings that SB attenuated certain types of self-administration and reinstatement, but not others, may be due to orexin's site of action during these behaviors. One possible site of action is the VTA, an area that receives dense projections from orexin neurons (Fadel and Deutch, 2002; Nakamura et al., 2000; Peyron et al., 1998). A recent study (Narita et al., 2006) showed

that microinjection of SB into VTA during conditioning reduced acquisition of a morphine CPP, indicating that orexin in VTA is critical for learning this stimulus-drug relationship. Further evidence that LH orexin projections to VTA are involved in learning stimulus-drug relationships comes from studies using bilateral neurotoxic lesions of LH neurons, or a unilateral neurotoxic lesion of LH neurons combined with injections of SB into the contralateral VTA just preceding each of 3 morphine CPP conditioning trials (Harris et al., 2007a). Results were similar with both manipulations, and are illustrated for the contralateral disconnection procedure in Fig. 5. Both of these manipulations prevented learning the CPP, as evidenced by no preference expressed on the subsequent CPP test day. Normal preference for the morphine-paired side appeared in animals in which either a neurotoxic lesion was outside of LH, or the contralateral microinjection of SB was outside of VTA. These results are consistent with those of other recent studies (Narita et al., 2006), and indicate that LH orexin is not only involved in conditioned reward processes, but also in plasticity in the VTA associated with learning at least some types of stimulus-drug relationships.

To test whether orexin is also necessary for the acquisition of cocaine-associated cues during self-administration, a Pavlovian conditioned-cues paradigm was utilized, as previously described by See and colleagues (See, 2005). In this paradigm, animals were first trained to self-administer cocaine (0.2 mg/infusion) in the absence of cues. After five days of stable responding, animals were exposed to a single Pavlovian conditioning session, in which no levers were extended and the animals received passive infusions of cocaine paired with discrete tone and light cues. The number of infusions was based on the self-administration for each animal in previous sessions. The Pavlovian session was followed by five more days of self-administration without cues. Following extinction of lever pressing in the absence of cocaine, drug-seeking was robustly reinstated when active lever presses produced the cocaine-paired cues alone. Interestingly, administration of 30 mg/kg SB prior to the Pavlovian acquisition session had no effect on subsequent cue-elicited reinstatement of lever responding (Smith et al., in press). That is, animals pretreated with SB or vehicle prior to the Pavlovian conditioning session showed similar reinstatement responding, indicating that signaling at OxR1 was not necessary for acquiring cocaine-cue associations in this paradigm. In contrast, 30 mg/kg SB in these same animals significantly reduced the expression of conditioned-cue-elicited drug-seeking when administered just prior to a subsequent reinstatement session (Smith et al., in press). This is consistent with the results described above showing that cue-induced reinstatement of cocaine-seeking requires OxR1 transmission.

Together, these experiments indicate that orexin is necessary for learning morphine-stimulus associations and preference, but not for learning the cocaine-stimulus relationships required for cue-evoked reinstatement of cocaine-seeking behavior. There are several procedural differences between these two learning paradigms that might underlie these different results for SB administration, including CPP vs. self-administration. However, these findings may also be due to differences between cocaine and morphine, and where orexin acts to modulate reward-seeking. These possibilities will be discussed in detail below in the Discussion section.

Altered hedonic processing during protracted abstinence: possible role of LH orexin neurons

Chronic exposure to cocaine or morphine, followed by protracted forced abstinence, results in dramatically altered preferences for drug and natural rewards. Thus, preference for morphine or cocaine is increased, and for food or novelty is decreased, at 2 or 5 weeks following protracted forced abstinence from chronic morphine or cocaine (Aston-Jones and Harris, 2004a; Harris et al., 2001; Harris and Aston-Jones, 2001). This increased preference for drug, and decreased interest in natural rewards, is similar to reports by addicts (Jaffe, 1990), and

could be a source of difficulty in maintaining prolonged abstinence from drugs after previous chronic exposure.

In CPP studies, examination of Fos expression on the preference test day revealed neural substrates related to this altered hedonic processing during protracted abstinence. Those brain areas with altered Fos that mirrored altered preferences for drug and natural rewards were hypothesized to be particularly good candidates for underlying altered reward processing, i.e., brain areas where Fos expression was higher than normal when withdrawn animals were tested for drug preference, and where Fos expression was lower than normal when withdrawn animals were tested for food or novelty preference. These studies revealed three such areas: the basolateral amygdala, nucleus accumbens shell, and LH (Aston-Jones and Harris, 2004a). Notably, the LH region that contained altered Fos expression in this study coincided with the area that contains orexin neurons. Subsequent analysis of LH sections for both Fos and orexin revealed that, indeed, Fos activation in orexin neurons in LH was higher than normal for animals in protracted morphine abstinence tested for drug preference, but lower than normal for animals in protracted morphine abstinence tested for food preference – i.e., the Fos expression in LH orexin neurons closely mirrored the altered preferences that resulted from protracted drug abstinence (Fig. 6)(Aston-Jones and Harris, 2004a). This result is consistent with the findings that Fos expression in LH orexin neurons correlates closely with preferences for morphine, cocaine or food reward (Harris et al., 2005), and indicates that LH orexin neurons may play a significant role in the altered hedonic processing that occurs during protracted drug abstinence. Consistent with this view is the fact that many of these Fos-activated orexin neurons project to VTA (described below), and that orexin mRNA expression is changed after opiate, ethanol, or cocaine exposure (Georgescu et al., 2003; Lawrence et al., 2006; Zhou et al., 2006; Zhou et al., 2008). Other neural systems in addition to LH orexin neurons may be involved in this altered hedonic processing during protracted abstinence as well (Aston-Jones and Harris, 2004b; Harris and Aston-Jones, 2007).

Functional dichotomy of the orexin system

Functional differences for LH versus DMH/PeF orexin neurons

As reviewed above, orexin neurons appear to be involved both in arousal and in reward-seeking, and evidence indicates that orexin neurons are functionally dichotomous (Harris and Aston-Jones, 2006). Thus, reward-seeking functions are associated primarily with orexin cells in LH, whereas arousal- and stress-related processes are more associated with orexin neurons in the DMH and PeF. For example, Estabrooke et al (Estabrooke et al., 2001) reported that PeF and DMH, but not LH, orexin neurons are Fos-activated during waking compared to sleep. Fadel et al. (2002) found that neuroleptics that cause weight gain preferentially activate LH, rather than more medial, orexin neurons, consistent with LH involvement in reward processes. In addition, morphine withdrawal activates Fos in DMH and PeF but not in LH orexin neurons (Sharf et al., 2008), whereas chronic ethanol consumption increased the area of orexin mRNA expression in LH, but not in more medial hypothalamic areas (Lawrence et al., 2006). Footshock stimulation induced Fos in PeF and DMH, but not in LH, orexin neurons (Harris et al., 2005), which may indicate that the role of orexin reported in stress-induced reinstatement of cocaine-seeking by Boutrel et al. (2005b) involves activation of norepinephrine (NE) and corticotropin-releasing factor (CRF) neurons by stress-responsive orexin neurons in DMH and PeF, but not by LH orexin cells (Harris and Aston-Jones, 2006).

The dichotomy of orexin function implies that orexin neurons differ in their input-output connections according to reward seeking vs. arousal. There is evidence to support this possibility: Fadel and colleagues found that orexin cells that project to VTA and medial prefrontal cortex (mPFC) originate preferentially from the LH (Fadel et al., 2002; Fadel and Deutch, 2002). Yoshida and colleagues reported that PeF/DMH orexin neurons are innervated

by other hypothalamic regions involved in homeostatic and arousal-related drive states, whereas LH orexin neurons are preferentially targeted by brainstem areas involved in autonomic and visceral processing, and by reward-related areas such as VTA and nucleus accumbens shell (Yoshida et al., 2006a). This possibility is currently being further examined (Richardson et al., 2007; Sartor and Aston-Jones, 2008); some results are briefly reviewed below.

Functional differences for OxR1 versus OxR2

A dichotomy in orexinergic involvement in both reward-seeking and arousal also may be related to differences in signaling at OxR1 and OxR2. The two receptors have different distributions of expression (Kilduff and de Lecea, 2001; Marcus et al., 2001; Trivedi et al., 1998), as well as different G protein coupling mechanisms (Zhu et al., 2003) and transmitter selectivity (Sakurai et al., 1998). Signaling at OxR2 has been hypothesized to be primarily related to arousal-related functions of the orexin system (Akanmu and Honda, 2005; Marcus et al., 2001; Willie et al., 2003), based, in part, on the finding that loss of signaling at OxR2 in particular is associated with symptoms of narcolepsy in animals (Lin et al., 1999; Willie et al., 2003). Further, whereas the OxR1 antagonists SB-334867 and SB-408124 caused no change to sleep-wake states, the OxR2 antagonist JNJ-10397049 significantly decreased latency to non-REM sleep and increased sleep time (Dugovic et al., 2009; Smith et al., 2003). In reinstatement studies, the OxR2 antagonist 4-pyridylmethyl (*S*)-*tert*-leucyl 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (4PT) did not block cue-induced reinstatement, in contrast to the attenuation effect seen with SB (Smith et al., in press). However, 4PT caused significantly greater impairments to locomotor activity than SB. Similarly, Wang et al. (2009b) found that cocaine-seeking was reinstated by local VTA microinjections of orexin A (non-selective agonist for OxR1 and OxR2), but not orexin B (selective agonist for OxR2). These data support the hypothesis that signaling at OxR2 is involved in arousal, whereas signaling at OxR1 is involved in reward-seeking.

Addiction-related inputs / outputs of orexin neurons

Orexin neurotransmission in VTA drives reinstatement

The orexin system projects widely throughout the CNS, so there are many possible targets where orexin might be acting during drug seeking and reinstatement. One site that seemed likely was the VTA, where dopamine (DA) neurons that play a critical role in reward and reinforcement mechanisms are located. Microinjections of orexin directly into VTA robustly reinstated morphine preference in animals that had previously been extinguished (Harris et al., 2005), and intra-VTA orexin injections elicited a CPP (Narita et al., 2007). Intra-VTA orexin A also reinstated extinguished cocaine-seeking in the self-administration paradigm (Wang et al., 2009b). Additionally, as discussed earlier, unilateral LH lesion combined with contralateral injection of SB into VTA blocked acquisition of morphine CPP (Harris et al., 2007a). Together with the preceding results, these findings provide strong evidence that orexin projections from LH to VTA play an important role in expression of drug preference, and may also be involved in relapse of drug-seeking following extinction.

Functional topography of orexin projections to VTA

The functional dichotomy reviewed above also implies that subpopulations of orexin neurons differ in their projection targets, so that some are involved in arousal whereas others are more concerned with reward and reinforcement. Ongoing studies have begun to address this issue using tract-tracing to retrogradely label orexin neurons from various targets, combined with Fos to identify orexin neurons that are activated by a CPP preference test in previously-naïve or morphine-dependent animals (Richardson and Aston-Jones, 2008; Richardson et al., 2007). Rats received a unilateral injection of the retrograde tracer wheat germ agglutinin-

colloidal gold (WGA-Au) in VTA. Animals were made morphine-dependent for 2 weeks by subcutaneously implanting two morphine pellets 7 days after WGA-Au microinjections; other rats were given placebo pellets. CPP conditioning began two weeks after pellet removal; conditioning and testing were identical to previously published reports for morphine (Aston-Jones and Harris, 2004a; Harris and Aston-Jones, 2003a; Harris and Aston-Jones, 2003b). Results showed that dependent animals exhibited an enhanced preference for the morphine-associated environment on the CPP test day compared to placebo-pelleted rats, as in previous studies (Harris and Aston-Jones, 2003d). A higher percentage of LH orexin neurons that exhibited Fos were retrogradely labeled from VTA in dependent than in non-dependent animals (26.4 ± 4.0% versus 11.4 ± 5.4%, $p<0.05$). The number of Fos-activated LH orexin neurons that project to VTA was significantly correlated with the intensity of conditioned preference in dependent animals ($R=0.743$, $p<0.05$). In addition, a greater percentage of retrogradely labeled orexin neurons were Fos-activated in dependent than in non-dependent animals (27.2 ± 4.0% versus 14.9 ± 5.0%, $p=.06$, $n = 6$ per group); this difference was not found for non-retrogradely-labeled neurons. In addition, the number of VTA-projecting orexin neurons that were Fos-activated was significantly correlated with the intensity of conditioned preference in dependent animals ($R=0.740$, $n=6$/group, $p<0.05$). Thus, as preference scores increased in dependent rats, the percentage of Fos-activated, VTA-projecting orexin neurons increased. There was no such correlation with preference found for Fos+ LH orexin neurons that were not retrogradely labeled from VTA, nor was this correlation significant in non-dependent animals.

This study demonstrates that Fos activation in VTA-projecting LH orexin neurons correlates with the intensity of reward during protracted abstinence, and supports the view that the VTA is an important site of action for orexin in reward processing and drug abuse. This study confirms the previous finding that prior morphine exposure enhances the preference for drug-associated environments (Harris and Aston-Jones, 2003b), and shows that animals that undergo prolonged forced abstinence have a higher degree of activation in LH orexin neurons that project to the VTA than control animals.

Related studies found that this phenomenon is exclusive to LH orexin neurons and that activation of VTA-projecting orexin neurons in DMH or PeF does not correlate with morphine preference after prolonged forced abstinence (Richardson and Aston-Jones, 2008). In animals that underwent CPP conditioning during protracted morphine abstinence (post-dependent subjects), the percentage of VTA-projecting LH orexin neurons that were Fos+ was positively correlated with the amount of conditioned preference ($r=0.743$, $p<0.05$, $n=6$). In contrast, no such correlation was observed for the percentage of VTA-projecting orexin neurons that were Fos+ in the PeF ($r=0.044$ morphine, $r=-0.411$ placebo) or DMH ($r=-0.24$ morphine, 0.434 placebo).

The preceding results were obtained using microinjections of WGA-Au into caudal VTA (at or caudal to interpeduncular nucleus), an area shown to be preferentially activated by orexin (Vittoz et al., 2008). However, VTA is functionally and anatomically heterogeneous (Carlezon et al., 2000; Carlezon et al., 2001; Ikemoto et al., 1998), so it is important to determine whether orexin neurons that project to rostral VTA were also Fos-activated by morphine conditioning in post-dependent animals. Preliminary analyses revealed that the percentage of orexin cells that project to rostral VTA and were Fos-activated in post-dependent rats after morphine CPP was significantly greater than the percentage in non-dependent rats ($n=6$/group, $p=0.036$) (Richardson and Aston-Jones, 2009). This significant relationship was only evident in orexin neurons located in LH. The numbers of triple labeled cells in the DMH ($p=0.08$) and PeF ($p=0.28$) were not significantly different in post-dependent versus non-dependent rats. These results indicate that LH orexin neurons that project to either rostral or caudal VTA are activated by stimuli previously associated with morphine during the post-dependent state. This elevated

orexin input to VTA may contribute to the propensity for relapse during protracted abstinence in addicts. Future studies will determine if the activation of LH orexin neurons that project to other areas less directly linked with reward (e.g., locus coeruleus, tuberomammillary nucleus) is also enhanced after morphine preference testing.

Activation of LH vs. DMH/PeF orexin neurons differentially affects VTA neurons

As noted above, previous work indicates that LH and DMH/PeF neurons may contribute differentially to reward-seeking behavior. To investigate potential mechanisms of this differential contribution, we recorded from neurons in the VTA of anesthetized rats while infusing the Y4 agonist rPP (which, as noted above, preferentially activates orexin neurons) into either the LH or DMH/PeF orexin fields (Massi et al., 2007). The results support a dichotomy of function for anatomically separate orexin neurons and suggest that VTA may be one place where this dichotomy is evident. Stimulation of LH with rPP mainly inhibited putative DA neurons (37%) and activated putative GABA neurons (25%). In contrast, rPP injection in DMH/PeF most frequently activated DA neurons (16%) and inhibited GABA cells (27%). These responses were blocked by local application of SB into VTA. In addition, the inhibition of DA neurons by LH stimulation was changed to excitation after intra-VTA microinjection of the GABA-A antagonist picrotoxin, indicating that LH orexin projections may indirectly inhibit DA neurons via local GABA interneurons. Notably, cells that responded to LH stimulation were usually not responsive to DMH/PeF stimulation, and vice versa, indicating differential targeting of VTA neurons by projections from these two orexin cell fields. Thus, the net effect of orexin neural activity on VTA DA neurons may be strongly regulated by local interneurons that mediate feed-forward inhibition in addition to direct activation of DA neurons by orexin. If these interneurons are in an excitable state, then orexin neuronal activity may inhibit DA neurons via this feedforward inhibition. Conversely, if the interneurons are not excitable then orexin neural activity would be expected to activate DA neurons by the direct excitatory pathway. The behavioral function of this mixed direct and feedforward pathway from LH orexin to VTA DA neurons is unknown. One possibility is that activation of certain DA neurons potentiates specific reward-directed behavior, whereas inhibition of other DA neurons suppresses competing reward-related behavior. Further studies are needed to test this and other possibilities.

In addition to these direct effects of orexin on neurons in VTA, recent studies have also found that orexin can potentiate glutamate responses of VTA DA neurons.

Orexin modulates glutamate responses in VTA DA neurons

Dopaminergic neurons of VTA are strongly implicated in regulating drug-seeking (Hyman et al., 2006), and activation of these cells via glutamate transmission is important for several aspects of drug abuse. Repeated cocaine causes release of glutamate in VTA (Kalivas and Duffy, 1998), and reinstatement of drug-seeking elicited by cues, context, stress, or cocaine is dependent on glutamate inputs to VTA (Bossert et al., 2004; Sun et al., 2005; Wang et al., 2005; Wise, 2009; You et al., 2007). Work from our laboratory showed that glutamate transmission and plasticity in VTA is important for learning a cocaine CPP, and for learning or expressing a morphine CPP (Harris and Aston-Jones, 2003c; Harris et al., 2004). A single injection of cocaine induces long-term potentiation (LTP) in VTA DA neurons that is dependent upon NMDA receptor activation (Borgland et al., 2004; Borgland et al., 2006; Thomas and Malenka, 2003; Ungless et al., 2001), supporting the hypothesis that excitation-dependent plasticity in VTA is critical in regulating drug-related behaviors. A recent study extended these results by showing that repeated cocaine injections produced larger LTP in DA neurons than a single injection (Liu et al., 2005), indicating that the stronger abuse potential with repeated cocaine experience may result in part from DA neuronal plasticity.

Orexin appears to interact with glutamate function in VTA DA neurons, and this interaction may be significant for drug abuse behavior. Borgland et al (2006) demonstrated that cocaine-induced glutamatergic plasticity in VTA DA neurons depends critically upon orexin inputs. By co-administering the OxR1 antagonist SB with cocaine, the investigators blocked the glutamate-dependent LTP in midbrain DA neurons described in the studies above. They also showed that orexin administration to midbrain slices produced a late-phase glutamate-dependent LTP in DA neurons, due primarily to the insertion of new NMDA receptors in the synapse that facilitated an AMPA receptor-mediated LTP. Supporting the relationship between VTA synaptic plasticity and cocaine abuse, they further demonstrated that SB injected either systemically or directly into the VTA blocked the development of locomotor sensitization following repeated cocaine injections. These findings provide important new information indicating that orexin in VTA plays an important role in synaptic plasticity of DA neurons.

Orexin modulates responses of VTA DA neurons to PFC activation

As described above, glutamate inputs and plasticity in VTA are essential for learning stimulus-drug associations (Harris and Aston-Jones, 2003c; Harris et al., 2004), and orexin increases responses and plasticity in VTA DA neurons evoked by glutamate inputs (Borgland et al., 2006). However, the specific glutamate inputs that are modulated by orexin have not been described. Glutamatergic afferents to VTA originate from several sources, including PFC, BNST, subthalamic nucleus and pedunculopontine tegmental nucleus (Carr and Sesack, 2000; Geisler and Zahm, 2005; Georges and Aston-Jones, 2002; Kita and Kitai, 1987; Rinvik and Ottersen, 1993; Sesack and Pickel, 1992). It seems plausible that brain regions representing stimulus information that becomes associated with drug administration might provide glutamate inputs to VTA that would be modulated by orexin, providing a neural substrate for orexin-dependent conditioned behaviors in addiction.

Inputs from mPFC to VTA are important to the function of the DA system, including its role in reward and drug abuse. The mPFC provides direct glutamate innervation of DA and GABA neurons in VTA (Carr and Sesack, 2000; Sesack et al., 1989; Sesack and Pickel, 1992; Sesack et al., 2003), and regulates the release of DA in nucleus accumbens (Karreman and Moghaddam, 1996; Taber et al., 1995; You et al., 1998), which plays a central role in drug abuse (Kalivas and McFarland, 2003; Kalivas and Volkow, 2005; Koob, 1999; Wolf et al., 2004). Indeed, recent work has shown that the strongest glutamatergic projection to rodent VTA originates in mPFC (Geisler et al., 2007). The mPFC is an important region for regulating goal-directed behaviors and impulse control. It receives highly processed information from several brain areas, and is involved in complex cognitive and behavioral processes (Bubser and Schmidt, 1990; Granon et al., 1994; Kolb, 1984; Muir et al., 1996; Watanabe, 1996). The mPFC has also been shown to be important in drug abuse and, specifically, in reinstatement of cocaine-seeking behavior (Kalivas and McFarland, 2003; McFarland and Kalivas, 2001; McFarland et al., 2004). Human functional imaging studies have revealed decreased metabolic activity in mPFC during drug withdrawal (Goldstein and Volkow, 2002) and increased activity in mPFC following exposure to drug cues (Childress et al., 1999; Grant et al., 1996; Maas et al., 1998). In the rat, single neurons in mPFC (in areas shown to project to VTA) are activated during both cocaine and heroin self-administration (Chang et al., 1997a; Chang et al., 1997b; Chang et al., 1998). Therefore, mPFC afferents to VTA are a potential source of VTA glutamate that is involved in reinstatement, learning and expression of drug-seeking behavior. Because results indicate that orexin neurons are stimulated by reward-associated cues (Harris et al., 2005), it seems possible that orexin will be released onto VTA DA neurons at about the same time that mPFC inputs representing stimulus-reward associations are arriving at these cells. Thus, the conditioned orexin input from LH may augment responses to glutamate (NMDA receptor-mediated) inputs from mPFC in a manner similar to that described by Borgland et al. (Borgland et al., 2006), serving to increase responding of VTA DA neurons to mPFC inputs.

The influence of orexin on prefrontal projections to the VTA was tested by recording the activity of DA neurons in isoflurane-anesthetized rats (Moorman and Aston-Jones, 2007). Responses were evoked from DA neurons by stimulating the prelimbic/infralimbic areas of the mPFC. We delivered orexin A directly to the site of the recorded neuron by using a second pipette glued to the recording pipette (Akaoka et al., 1992; Georges and Aston-Jones, 2002). Different combinations of microstimulation and orexin delivery during recording were used to ascertain the influence of orexin on mPFC-evoked responses in DA neurons.

Stimulation of the prelimbic/infralimbic regions in mPFC in the absence of orexin evoked short-latency (<50 ms) responses in 40% of putative DA neurons. Approximately 22% of DA neurons exhibited short-latency evoked responses within the range of monosynaptic projections (<25 ms (Thierry et al., 1983)). Orexin A (1.4 μM, 60 nl) applied directly to recorded neurons (in the absence of mPFC stimulation) produced strong increases in firing rate (in 58% neurons) and bursting (in 28% neurons) (Moorman and Aston-Jones, 2007), consistent with more global applications of orexin in slice (Korotkova et al., 2003) and *in vivo* (Muschamp et al., 2007). Local application of the OxR1 antagonist SB at 10 uM had little if any effect on spontaneous activity in DA neurons, and local SB at 100 μM modestly but significantly decreased activity in these cells, indicating that tonic orexin release may play some role in baseline impulse activity of DA neurons in the anesthetized preparation.

A major focus of these studies was to test whether orexin application would facilitate mPFC-evoked responses in DA neurons, possibly indicating a circuit substrate for orexin-induced facilitation of glutamate responses, or plasticity, in VTA involved in stimulus-drug conditioning. Therefore, the influence of mPFC stimulation on DA neurons was examined when given before, during or following local orexin application. Results showed that for most cells mPFC-evoked responses following orexin application were enhanced. An example of such an orexin-enhanced mPFC-evoked response is shown in Fig. 7. In contrast, an equivalent number of neurons exhibited enhanced and diminished mPFC-evoked responses (45% each) when tested during orexin application. The effect of OxR1 antagonism had the opposite effect: mPFC stimulation-evoked short-latency responses were decreased or abolished following application of SB (in 43% neurons), as were long-latency evoked responses (in 69% neurons), strengthening the argument for a relationship between orexin receptor activation and enhancement of mPFC-evoked responses.

It is likely that mPFC inputs are not the only excitatory projections to VTA facilitated by orexin. Several other areas send (in some cases relatively strong) glutamatergic projections to the VTA, including the LH (Geisler et al., 2007). Indeed, it has been shown that orexin neurons co-express orexin and glutamate (Rosin et al., 2003; Torrealba et al., 2003); the co-release of these transmitters could produce orexin-enhanced excitatory drive from the LH itself. The influence of orexin on other glutamatergic inputs remains to be tested. However, the complex stimulus information relayed to VTA from mPFC, and therefore the orexin-facilitated mPFC drive on dopamine neurons, may be particularly relevant for learning some types of stimulus-drug associations and for stimulus-induced reinstatement of extinguished drug-seeking. This relationship may underlie the observations that activation of both the mPFC and the LH orexin system are critical in learning stimulus-drug associations and in reinstatement of drug-seeking.

Combined with results described above demonstrating direct excitatory and indirect inhibitory effects of LH orexin stimulation on DA neural activity, these findings indicate a complex role for the influence of LH orexin on regulation of the DA system. We speculate that LH orexin neurons may inhibit non-specific activation of DA neurons (via GABAergic interneurons) while simultaneously facilitating the influence of certain inputs (e.g., mPFC-mediated conditioned responses) to specific DA neurons. The net result of this may be enhanced selectivity of DA neuron activity in certain behavioral contexts (e.g., reward-seeking). These

results are in line with work demonstrating important roles of orexin, mPFC, and DA in reward- and drug-seeking.

LH orexin afferents activated during cocaine place preference

Previous work in our lab revealed that LH orexin neurons show increased activity (indexed by Fos) during cocaine or morphine CPP, and orexin input to the VTA is necessary for the acquisition of morphine preference (Harris et al., 2005; Harris et al., 2007b). However, which inputs activate LH orexin neurons during drug-seeking behaviors remains unknown. We hypothesize that afferents that are importantly involved in conditioned activation of LH orexin neurons during cocaine CPP will show increased activity (indexed by Fos) during the expression of cocaine preference. Based on the functional and anatomical dichotomy of orexin neurons (Harris and Aston-Jones, 2006) (described above), we also believe that distinct afferents to the LH, as opposed to those to the PeF/DMH orexin cell field, will be activated during cocaine CPP expression. To test this hypothesis, the retrograde tracer cholera toxin b (CTb) was injected into the LH or PeF/DMH orexin field, and one week later animals were conditioned for cocaine CPP. Preliminary results (Sartor and Aston-Jones, 2008; Sartor and Aston-Jones, 2009) revealed that several brain areas have strong inputs to the LH orexin field (prelimbic cortex, infralimbic cortex, lateral septum, nucleus accumbens shell, bed nucleus of stria terminalis, and lateral preoptic area), consistent with previous findings (Sakurai et al., 2005; Yoshida et al., 2006b). However, only LH afferents from the lateral septum (LS) and ventral bed nucleus of the stria terminalis (vBNST) exhibited greater Fos activation during the expression of cocaine preference than saline-conditioned control animals (LS, 7.32 ±1.3% versus 15.0 ±1.7%, $p<0.05$; vBNST, 5.9 ±1.2% versus 18.8 ±3.6%, $p<0.05$). Notably, the percentages of CTb+ neurons that were also Fos+ in the LS and vBNST were positively correlated with the amount of preference exhibited on the CPP test day (LS, $R = 0.66$, $p<0.05$; vBNST, $R = 0.52$ $p<0.05$). Furthermore, LS and vBNST afferents to the LH, rather than PeF/DMH, were preferentially activated during cocaine preference (Sartor and Aston-Jones, 2008; Sartor and Aston-Jones, 2009).

These data indicate that afferents from vBNST and LS might be important in regulating LH orexin neurons during cocaine-seeking. Indeed, several publications have demonstrated a strong role for LS and vBNST in reward seeking. For example, the septal region was the first area reported to support operant responding via electrical self-stimulation (Olds and Milner, 1954). In addition, exposure to an environment that was previously associated with cocaine significantly increased Fos activity in LS (Brown et al., 1992; Franklin and Druhan, 2000), and acute or chronic exposure to cocaine markedly altered many electrophysiological characteristics of LS neurons (Lesse and Harper, 1985; Sheehan et al., 2004; Shoji et al., 1998; Simms and Gallagher, 1996). Likewise, BNST showed increased impulse activity following cocaine self-administration (Dumont et al., 2005), and is importantly involved in stress-induced reinstatement of cocaine seeking (Leri et al., 2002) and morphine withdrawal (Aston-Jones et al., 1999; Delfs et al., 1998). In addition, several studies have reported that vBNST and LS send strong projections to LH (Dong and Swanson, 2004; Risold and Swanson, 1997; Swanson and Cowan, 1979) and specifically LH orexin neurons (Sakurai et al., 2005; Yoshida et al., 2006b). Studies are currently underway to determine if LS and/or vBNST are necessary afferents to LH orexin during expression of cocaine CPP.

Discussion and hypothesis

The recent data reviewed above indicate that orexin is involved in conditioned behavioral responding to drug-associated stimuli. Cocaine self-administration studies found that OxR1 antagonism significantly attenuated cocaine-seeking elicited by stimuli previously associated with cocaine. SB reduced cue- and context-induced reinstatement of extinguished cocaine-

seeking, as well as relapse responding after 2 weeks of abstinence. On the other hand, SB had no effect on reinstatement of cocaine-seeking induced by cocaine itself, or on established cocaine self-administration. This indicates that orexin is uniquely involved in *conditioned* responding for cocaine-associated stimuli when cocaine is not present. Importantly, orexin is involved in drug-seeking following extinction or abstinence, and reinstatement induced by cues or context. Distinct neural pathways are responsible for these different types of drug-seeking (Fuchs et al., 2005; Fuchs et al., 2006b), which indicates that orexin may be part of a common mechanism for conditioned cocaine-seeking.

Why is the orexin system involved in stimulus-induced drug-seeking and opiate/alcohol/nicotine reinforcement but not priming and reinforcement for cocaine?

As reviewed above, the Ox1R antagonist SB reduced cue- and context-induced reinstatement of extinguished cocaine-seeking, as well as relapse after 2 weeks of abstinence. In addition, SB or other manipulations that interfere with orexin function attenuate alcohol and nicotine self-administration as well as cue-induced alcohol-seeking (Hollander et al., 2008; Lawrence et al., 2006; Richards et al., 2008), acquisition and expression of morphine preference (Harris et al., 2005; Harris et al., 2007b; Narita et al., 2006), and stress-induced reinstatement of cocaine- and alcohol-seeking (Boutrel et al., 2005a; Richards et al., 2008). In contrast, SB had no effect on reinstatement of cocaine-seeking induced by cocaine itself, on established cocaine self-administration, or on learning cocaine-stimulus associations (Smith et al., 2009a). SB effects on stimulus-induced drug-seeking and self-administration of non-psychostimulants but not on cocaine-induced drug-seeking and self-administration might indicate that the VTA is a critical site of orexin action for these conditioning effects. Orexin potentiates glutamate-mediated responses of VTA DA neurons (Borgland et al., 2006). Activation of VTA DA neurons is thought to be necessary for cocaine-seeking elicited by cues, context, stress, or abstinence (Crombag et al., 2002; Fuchs et al., 2006a; McFarland et al., 2004; See et al., 2001), which may explain why SB attenuates each of these behaviors. Additionally, ethanol and opiates increase extracellular DA via actions in VTA (Cami and Farre, 2003), which may explain why SB reduces ethanol self-administration (Lawrence et al., 2006; Richards et al., 2008) and morphine-stimulus conditioning (Harris et al., 2007a; Narita et al., 2006). In contrast, cocaine elicits DA release by acting directly on DA terminals, without requiring increased impulse activity of VTA DA neurons. Thus, the DA release (and consequent behavioral effects) evoked by cocaine during cocaine-induced reinstatement or cocaine-stimulus acquisition may bypass a critical target site of orexin actions, i.e., VTA somata and dendrites. This view indicates that the VTA is an important site of action for orexin in reward-associated functions. Consistent with this view are our recent findings that LH orexin neurons are more prominently involved in reward processes than are more medial orexin neurons in PeF and DMH (Harris and Aston-Jones, 2006). Also, we recently found that LH orexin neurons that project to VTA may be more strongly activated by reward-related stimuli than non-VTA-projecting LH or PeF/DMH orexin neurons (Richardson and Aston-Jones, 2008; Richardson and Aston-Jones, 2009), consistent with this hypothesis. Additional work tracing inputs and outputs of orexin neurons is underway to further define the topography of connections for reward-associated orexin neurons.

Although this hypothesis is consistent with many previous results, there are several caveats to this view and recent work by others indicate that VTA may not be the requisite site of orexin action for all reinstatement behaviors. Kenny and colleagues found that nicotine self-administration is attenuated by SB microinjection into insular cortex (Hollander et al., 2008). In addition, Wise and colleagues found that stress-induced reinstatement of cocaine-seeking was not attenuated by SB microinjected into VTA (Wang et al., 2009b; Wise, 2009). In addition, these investigators found that cocaine-induced reinstatement of cocaine-seeking does, in fact, require increased glutamate receptor stimulation and activation of DA neurons to

mediate the cueing properties of the cocaine prime (Wise et al., 2008). The manipulations used in this study (lidocaine or kynurenate infusion into VTA) may have prevented cocaine priming effects by decreasing spiking in DA neurons, which is required for cocaine-induced increase in DA release. However, if indeed glutamate input to VTA were needed for cocaine-primed reinstatement of seeking, then the above hypothesis would predict that cocaine-induced reinstatement should be sensitive to OxR1 antagonism by SB; this was not observed. Finally, the NE system, rather than the VTA DA system, may be the primary mechanism for the reinforcing effects of ethanol and opiates (Pierce and Kumaresan, 2006; Smith and Aston-Jones, 2008), although DA is necessary for initial learning of stimulus-conditioning. Orexins also may be acting in other structures known to be important for reinstatement, such as nucleus accumbens or extended amygdala, or through interactions with NE and CRF systems (Boutrel and de Lecea, 2008; Richards et al., 2008). Evidence supporting the latter comes from findings that reinstatement of cocaine-seeking induced by intracerebroventricular administration of orexin was blocked by NE and CRF antagonists (Boutrel et al., 2005b). Note, though, that this effect could be due to orexin inputs to NE or CRF neurons from DMH/PeF neurons rather than LH neurons. Interestingly, orexin and CRF systems have similar mechanisms of action in VTA (Bonci and Borgland, 2009), but appear to have largely independent actions in this region (Wang et al., 2009a). Future studies involving local administration of SB will be important to better elucidate the mechanisms for orexinergic involvement in the different forms of drug-seeking.

Orexin and food reward

Although most analysis of the orexin system to date has centered on drug reward, considerable evidence also implicates these neurons in food reward. Space constraints prohibit a full review of this topic, but some highlights include the finding that LH orexin neurons are strongly Fos-activated by food-associated CSs (Harris et al., 2005). Also, early studies showed that central orexin administration increases food intake, whereas food deprivation increases mRNA for orexin in hypothalamus (in fact, this observation prompted naming these peptides “orexins”, meaning appetite)(Sakurai et al., 1998). Moreover, administration of the Ox1R antagonist SB decreases food intake (Haynes et al., 2000; Rodgers et al., 2001), and recent studies show that this compound decreases consumption of high-fat food (Nair et al., 2008). In addition, consumption of high-fat food stimulated by mu receptor activation in the nucleus accumbens is associated with Fos induction in orexin neurons, and this stimulated feeding is inhibited by local SB injection into VTA (Zheng et al., 2007). Results from Petrovich et al (Petrovich et al., 2002) show that amygdala inputs to hypothalamus regulate cue-induced feeding in sated animals. Together, these sets of results indicate that orexin neurons may be involved in this circuit: amygdala may stimulate orexin neurons in response to food CSs, which in turn may play a role in conditioned overeating and obesity. Further studies are needed to evaluate this possibility. In any case, these results indicate that the orexin system is involved in reward processing for natural rewards such as food, in addition to drug rewards and addiction.

Orexin and reward: a role in drug-seeking and relapse

The results reviewed above indicate that LH orexin neurons are stimulated in proportion to drug or food preference, that stimulation of LH orexin neurons drives reinstatement of an extinguished preference for morphine, and that orexin neurotransmission is needed for stimulus-induced (but not for cocaine-induced) reinstatement of extinguished cocaine-seeking. These results indicate a role for orexin in drug-seeking and addiction. Interestingly, human narcoleptics, who have few or no orexin neurons, rarely exhibit stimulant abuse and seeking despite the fact that they are treated for years with stimulants (Sakurai, 2007). Together, these findings indicate that LH orexin neurons may be stimulated by cues associated with drug exposure, and that these neurons may be part of circuitry that is critically involved in drug abuse, and specifically in stimulus-induced drug relapse. Moreover, VTA might be a critical

region for orexin actions in reward-seeking behaviors. These findings are clinically significant, and indicate that development of compounds that specifically target LH orexin neurons, or orexin receptor actions in VTA DA neurons, may provide novel treatments for addiction and relapse. In particular, an OxR1 antagonist would reduce the propensity to relapse to drug-taking behavior, and would help maintain abstinence in addicts re-exposed to previous drug cues. Additional studies in this vein will advance our understanding of the role of orexin in addiction and lead to possible novel therapeutic targets.

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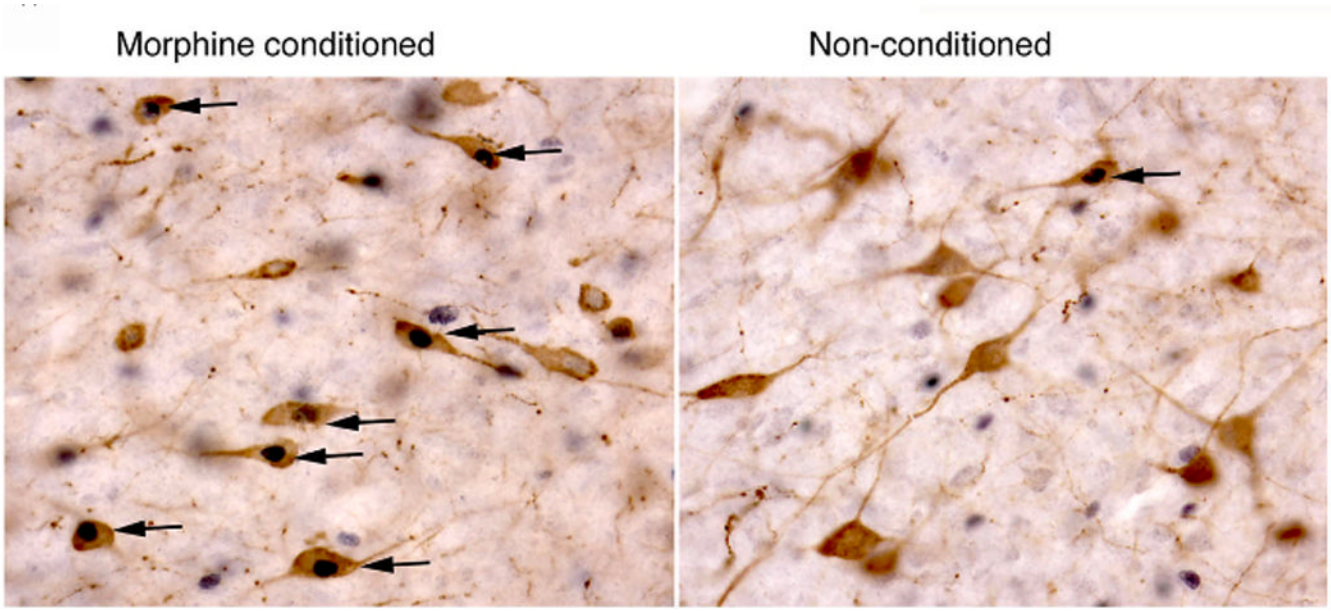

Figure 1.
High-power photomicrograph of the LH showing the double labeling of orexin (brown cytoplasm) and Fos protein (black nuclei) in morphine-conditioned and non-conditioned animals, as indicated. Black arrows indicate double-labeled cells. Taken from (Harris et al., 2005).

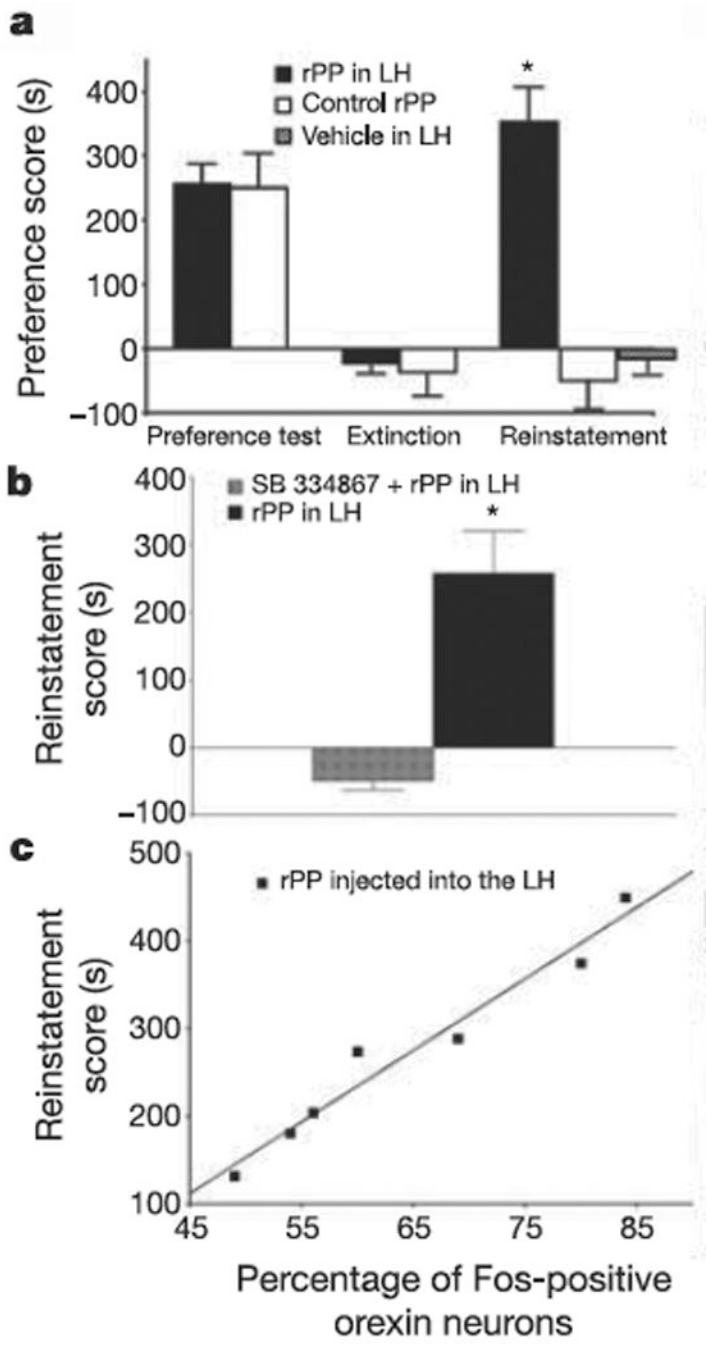

Figure 2.

Activation of LH orexin neurons by rPP reinstated an extinguished preference for morphine. a, Preference scores are shown for both rPP- (150 nM) and vehicle-injected groups (mean ± s.e.m. in morphine-paired side minus saline-paired side) during the initial conditioning test, after extinction and during the reinstatement test. b, The selective OxR1 antagonist, SB 334867 (20–30 mg kg), blocked reinstatement by rPP (n = 8). Data were included only if rPP injection into LH on the following day (without the antagonist pretreatment) produced reinstatement of preference. c, Plot of correlation between reinstatement scores and percentages of LH orexin neurons that were Fos activated in rPP reinstated animals. Taken from (Harris et al., 2005).

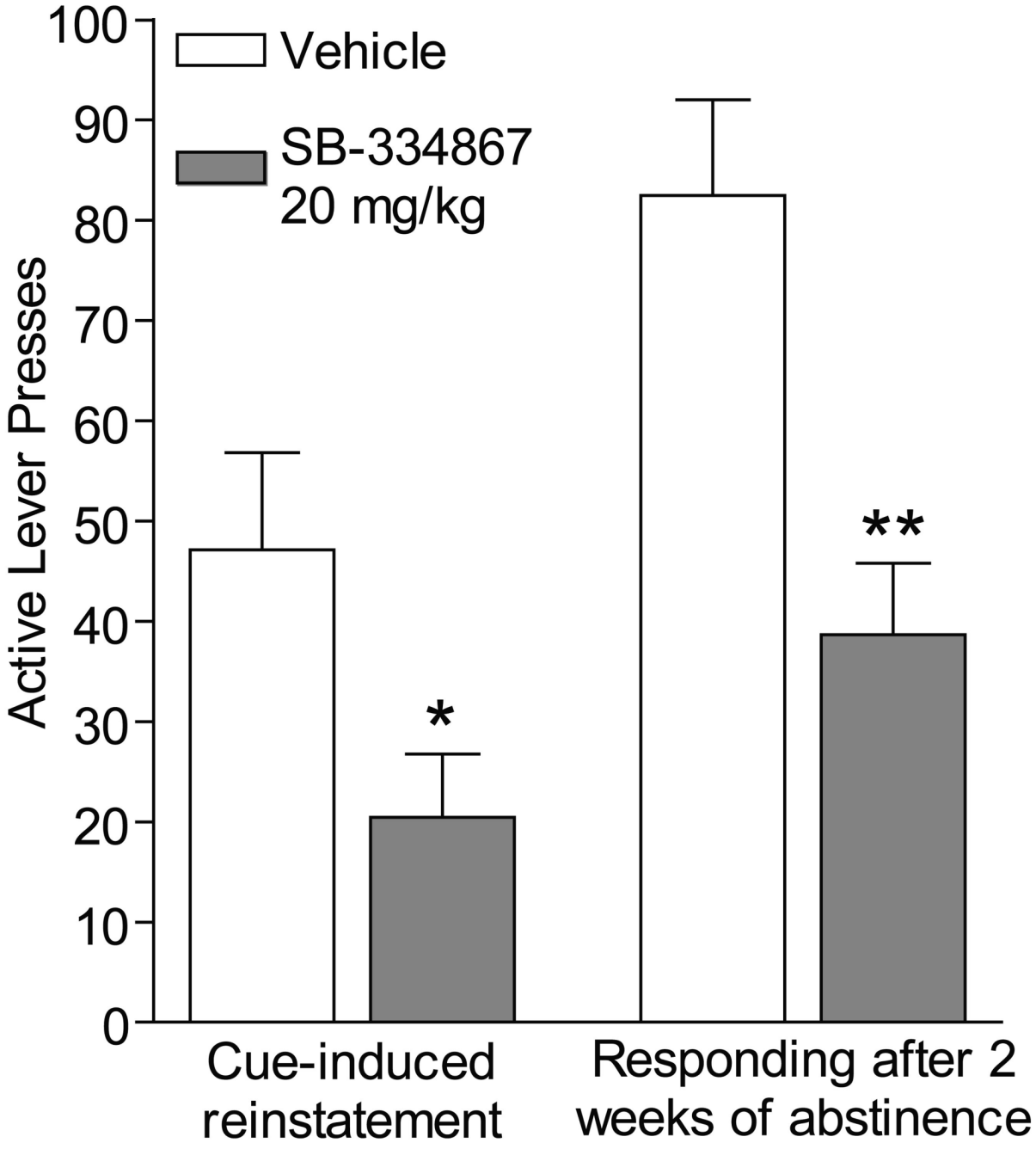

Figure 3.
SB-334867 attenuated cocaine-seeking elicited by cues or following abstinence. Pretreatment with SB-334867 (20 mg/kg, ip) significantly reduced cue-induced reinstatement of extinguished lever-pressing as compared to vehicle pretreatment in the same animals (*$p <$ 0.01). Pretreatment with SB-334867 also significantly reduced lever-pressing (cocaine-seeking) following 2 weeks of abstinence from cocaine self-administration as compared to animals given vehicle (**$p < 0.005$).

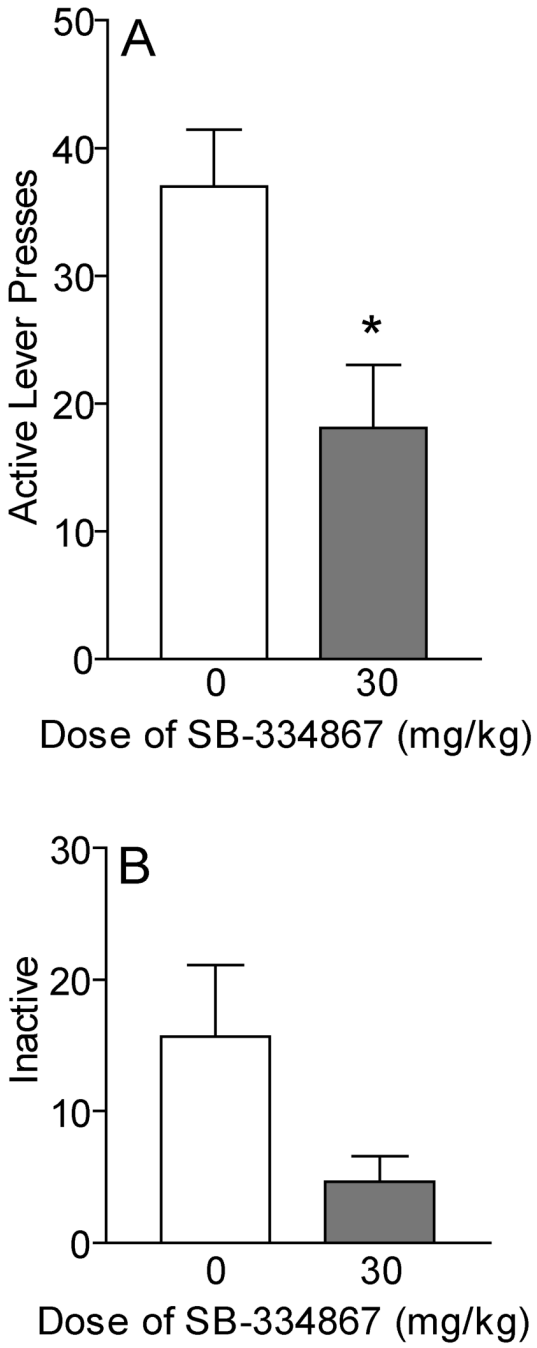

Figure 4.

SB-334867 (30 mg/kg, ip) attenuated reinstatement elicited by contextual stimuli in an ABA (renewal) design. Animals were trained to self-administer cocaine in a distinct context and then given extinction training in an alternate environment. In a within-subjects design, rats were pretreated with SB (30 mg/kg, i.p.) or vehicle prior to re-exposure to the original self-administration context. Mean (± SEM) number of presses on the active and inactive lever in a 2-hour reinstatement session in the self-administration chamber is shown. a, Active lever responding during context-induced reinstatement of extinguished cocaine-seeking was significantly attenuated by SB (n = 9), as compared to vehicle (*$p < 0.001$). b, Inactive lever

responding was not significantly affected by SB pretreatment. The apparent decrease in responding on the inactive lever is expected if SB attenuates drug-seeking.

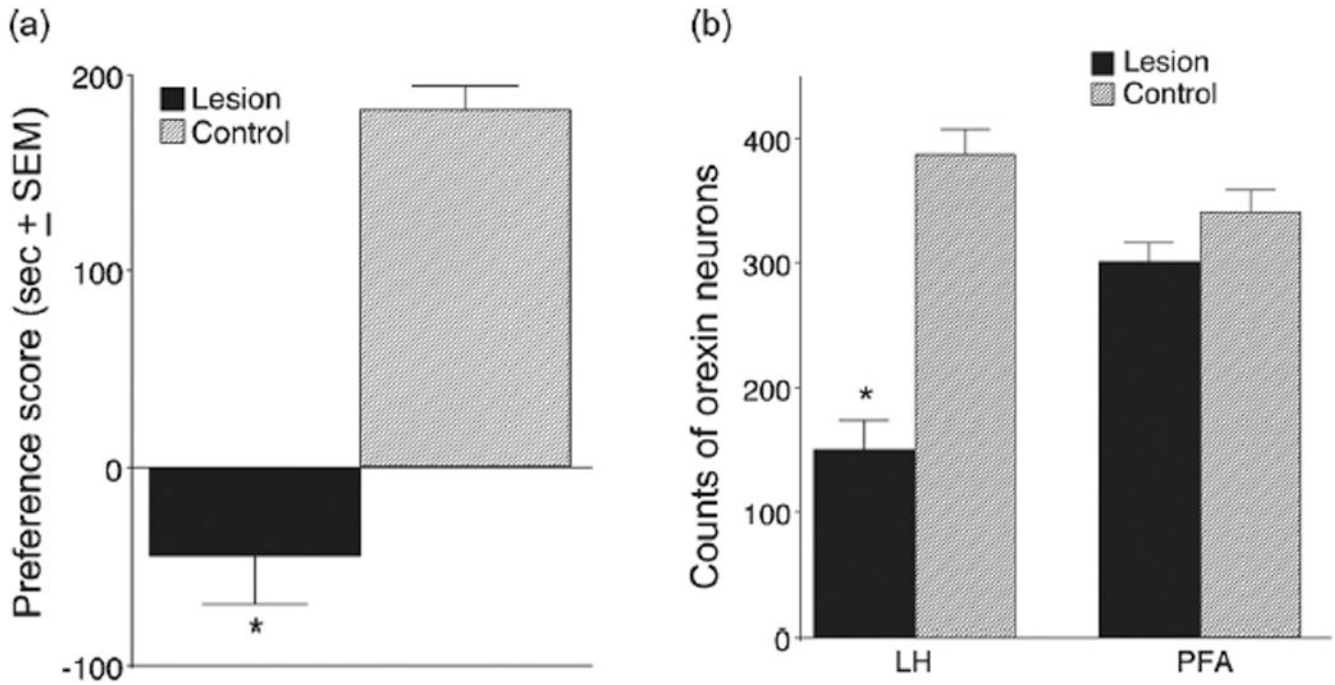

Figure 5.

(a) Conditioned place preference (CPP) scores and (b) orexin neuronal cell counts for animals given unilateral NMDA injections in the LH and microinjections of SB-334867 in the contralateral VTA during CPP training. (a) Preference scores were calculated by subtracting the time spent in the morphine-paired chamber during the preconditioning day from the time spent in that chamber on the test day (i.e., post-conditioning). Control animals received vehicle instead of NMDA in the LH and received the same SB injections in the contralateral VTA. Scores represent group mean ± SEM. (b) Neuronal cell counts of surviving orexin neurons in LH or neighboring PFA (aka PeF) from six adjacent 40 um-thick sections at the level of the neurotoxin injection from animals with effective lesions. Control refers to the number of orexin neurons found on the non-lesioned side in the same slices (*$P < .01$, $n = 9$). Taken from (Harris et al., 2007a).

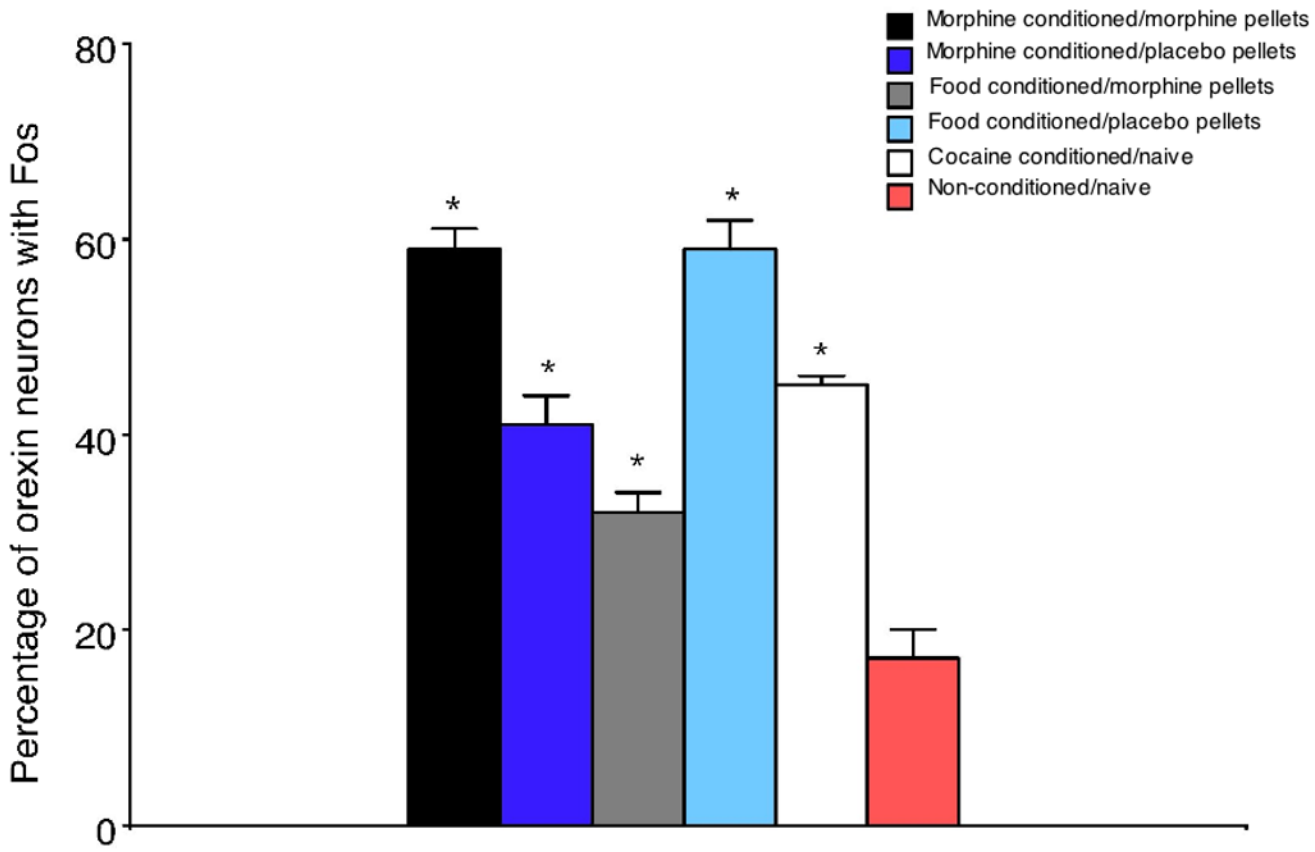

Figure 6.

Fos expression in LH orexin neurons as a function of treatments, as indicated. Morphine- and food-conditioned animals were given morphine or placebo pellets for 2 weeks, and pellets were removed and animals remained in their home cages for 5 weeks before CPP conditioning, as in our previous publications (Aston-Jones and Harris, 2004a; Harris and Aston-Jones, 2003d). Note that a higher percentage of LH orexin neurons exhibited Fos in withdrawn animals than in placebo-pelleted animals subjected to morphine CPP. Conversely, a lower percentage of LH orexin neurons exhibited Fos in withdrawn animals than in placebo pelleted animals subjected to food CPP. Cocaine CPP in naïve rats (no prior drug treatment) also increased Fos expression in LH orexin neurons. Taken from (Aston-Jones et al., 2009).

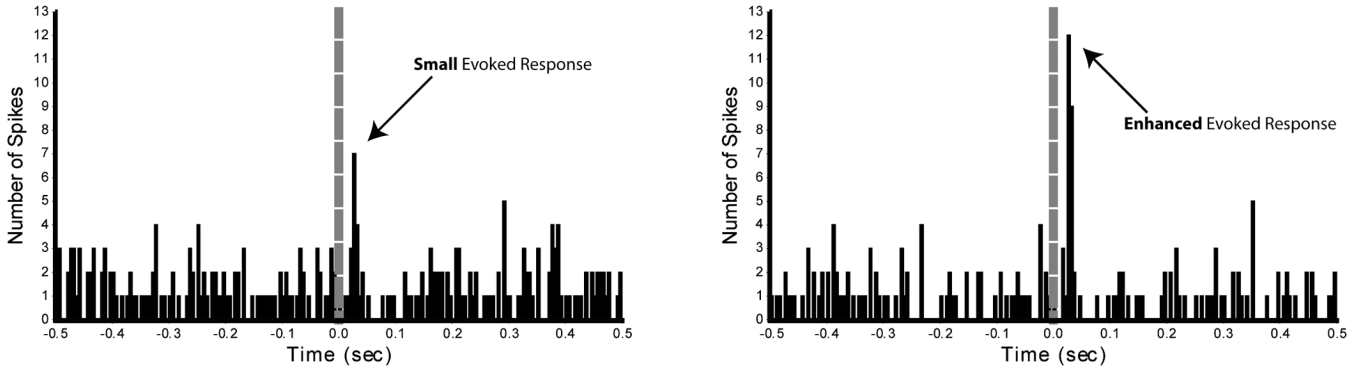

Figure 7.

Responses of a single DA neuron evoked with mPFC stimulation before and after application of orexin A to the recorded neuron. Left panel shows the responses of a DA neuron evoked with 1 mA stimulation of the mPFC. Right panel shows the responses of the same neuron to the same stimulation approximately 5 min following delivery of 60 nl of 1.4 μM orexin A locally onto the recorded neuron. Dashed line is the onset of stimulation. Taken from (Aston-Jones et al., 2009).

Table 1

Orexin-Fos double labeling

The percentages of orexin-positive cells that were also Fos-positive are indicated for each group in the LH, PeF and DMH. The right column gives correlation coefficients for the comparisons between these percentages and the corresponding preference score in each animal. LH by group ANOVA F(3,39)=33, p<0.01. The non-orexin Fos+ neurons in the LH are given as total counts not percentages.

Groups:	**Cell Types:**	**Percentage Fos+**	**Correlations R:**	
Morphine	**Orx LH**	**48±2***	**.72**	**p<.01***
Conditioned		55±6	.30	p=.34
N=12	NonOrx LH	62±2	.04	p=.91
	Orx PeF	67±4	−.11	p=.71
	Orx DMH			
Food	*Orx LH*	**50±3***	**.87**	**p<.01***
Conditioned		47±5	.20	p=.64
N=8	NonOrx LH	42±3	.26	p=.54
	Orx PeF	47±6	.16	p=.71
	Orx DMH			
Cocaine	*Orx LH*	**52±5***	**.90**	**p<.01***
Conditioned		78±7	.51	p=.20
N=8	NonOrx LH	67±3	.41	p=.32
	Orx PeF	74±3	.50	p=.20
	Orx DMH			
Non-	*Orx LH*	**17±2**	**.11**	**p= .81**
conditioned		43±6	.30	p=.53
N=15	NonOrx LH	52±4	.42	p=.36
	Orx PeF	59±4	.02	p=.96
	Orx DMH			
Naïve	*Orx LH*	**15±1**		
N=6		29±8		
	NonOrx LH	52±3		
	Orx PeF	57±6		
	Orx DMH			
Novelty	*Orx LH*	**18±2**	**.09**	**p=.86**
conditioned		50±1	−.52	p=.31
N=6	NonOrx LH	56±3	.02	p=.97
	Orx PeF	63±5	.42	p=.43
	Orx DMH			

* significantly different from other groups, p<0.05 Orx = orexin positive neurons. Taken from (Harris et al., 2005).