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Hypocretin / orexin involvement in reward and reinforcement

Rodrigo A. España

Department of Neurobiology and Anatomy, Drexel University College of Medicine, Philadelphia, PA, USA

Abstract

Since the discovery of the hypocretins/orexins, a series of observations have indicated that these peptides influence a variety of physiological processes including feeding, sleep/wake function, memory, and stress. More recently, the hypocretins have been implicated in reinforcement and reward-related processes via actions on the mesolimbic dopamine system. Although investigation into the relationship between the hypocretins and reinforcement/reward remains in relatively early stages, accumulating evidence suggests that continued research into this area may offer new insights into the addiction process and provide the foundation to generate novel pharmacotherapies for drug abuse. The current chapter will focus on contemporary perspectives of hypocretin regulation of cocaine reward and reinforcement via actions on the mesolimbic dopamine system.

Keywords

hypocretin; orexin; cocaine; addiction; self-administration; nucleus accumbens; conditioned place preference; dopamine; voltammetry; microdialysis

I. Introduction

Hypocretin peptide and receptors

The hypocretins (also termed orexins) were discovered in 1998 by two independent research groups (de Lecea *et al.*, 1998; Sakurai *et al.*, 1998), and consist of the hypocretin-1 and hypocretin-2 peptides which are also known as orexin A and B. These peptides are synthesized solely within a restricted portion of the lateral and posterior hypothalamus and perifornical areas (Chemelli *et al.*, 1999; Date *et al.*, 1999; de Lecea *et al.*, 1998; Peyron *et al.*, 1998; Sakurai *et al.*, 1998). While the hypocretin neurons are confined to a relatively circumscribed region, immunohistochemical studies demonstrate widespread projections of these neurons throughout the brain, suggesting varied actions of these peptides (Date *et al.*, 1999; Nambu *et al.*, 1999; Peyron *et al.*, 1998; van Den Pol, 1999).

To date, two G-protein-coupled hypocretin receptor subtypes have been identified. The hypocretin 1 receptor preferentially binds the hypocretin-1 peptide, whereas the hypocretin 2 receptor binds both hypocretin-1 and -2 peptides with equi-affinity (Sakurai *et al.*, 1998).

Both the hypocretin 1 and 2 receptors are expressed widely throughout the brain with slight, yet sometimes notable variations in distribution (Backberg *et al.*, 2002; Bourgin *et al.*, 2000; Cluderay *et al.*, 2002; Greco and Shiromani, 2001; Hervieu *et al.*, 2001; Marcus *et al.*, 2001; Suzuki *et al.*, 2002; Trivedi *et al.*, 1998). For example, hypocretin 1 receptors are observed throughout the cortex, hippocampus, paraventricular thalamic, ventromedial hypothalamic and arcuate nuclei, as well as the dorsal raphe, locus coeruleus, substantia nigra (SN) and ventral tegmental area (VTA). In contrast, hypocretin 2 receptors are observed in the cortex, septal nuclei, hippocampus, medial thalamic groups, the dorsal and median raphe nuclei, tuberomammillary nucleus, dorsomedial and paraventricular hypothalamic nuclei, SN and VTA (Backberg *et al.*, 2002; Bourgin *et al.*, 2000; Cluderay *et al.*, 2002; Greco and Shiromani, 2001; Hervieu *et al.*, 2001; Marcus *et al.*, 2001; Suzuki *et al.*, 2002; Trivedi *et al.*, 1998).

Although extensive evidence demonstrates that the lateral hypothalamus receives inputs from much of the central nervous system, only few advances have been made in elucidating the afferent inputs to the hypocretin neurons. Nevertheless, these studies have proven to be valuable at demonstrating that hypocretin neuronal activity is likely influenced by a diverse set of functionally distinct neural structures. For example, studies using classic retrograde and anterograde tracing methods, as well as more novel molecular techniques, demonstrate that hypocretin neurons are innervated by monoaminergic regions such as the dorsal raphe, VTA, and locus coeruleus (Abrahamson *et al.*, 2001; Fadel and Deutch, 2002; Horvath *et al.*, 1999; Sakurai *et al.*, 2005; Yoshida *et al.*, 2006). Although it is generally unclear as to whether these inputs exert excitatory or inhibitory actions, it is evident that hypocretin neurons receive signals from regions that govern motivation, homeostatic drive, behavioral state, and autonomic tone, and thus the hypocretin neurons are poised to regulate a variety of neural functions.

Hypocretin regulates arousal and arousal-related behavioral activity

Despite initial connections between hypocretins and feeding, substantial research now suggests that the hypocretin system plays a critical role in the regulation of arousal, including processes involved in governing sleep and waking. For example, hypocretin neurons are preferentially active during periods of alert waking, novelty, and behavioral arousal, and are responsive to wake-promoting drugs (España *et al.*, 2003; Estabrooke *et al.*, 2001; Lee *et al.*, 2005; Mileykovskiy *et al.*, 2005). Further, hypocretin-immunoreactive fibers and hypocretin receptors are located within many arousal-related structures including the noradrenergic, serotonergic, histaminergic, and dopaminergic systems (Brown *et al.*, 2001; Fadel and Deutch, 2002; Hagan *et al.*, 1999; Horvath *et al.*, 1999). Consistent with this, hypocretin levels fluctuate across the day/night cycle with the highest levels observed during the dark-phase when rats are typically awake (Yoshida *et al.*, 2001). Further, intracerebroventricular (i.c.v.) infusions of hypocretin-1 or hypocretin-2 dose-dependently increase waking and suppress sleep (España *et al.*, 2001; España *et al.*, 2002; Hagan *et al.*, 1999; Ida *et al.*, 1999; Piper *et al.*, 2000) and elicit behaviors typical of spontaneous waking and/or high arousal conditions, including those associated with motivated states. Similar wake-promoting and behavioral-activating effects are also observed when hypocretin-1 is focally delivered to the VTA, locus coeruleus, basal forebrain, and other arousal-related

regions (Bourgin *et al.*, 2000; España *et al.*, 2001; Hagan *et al.*, 1999; Vittoz and Berridge, 2006).

Additional evidence for hypocretin involvement in arousal processes comes from clinical and animal studies indicating an important link between dysregulation of hypocretin neurotransmission and the sleep/arousal disorder narcolepsy. Numerous studies using people with narcolepsy have demonstrated that these patients have reduced circulating levels of hypocretin in their cerebrospinal fluid and commonly display a degenerative loss of hypocretin neurons (Blouin *et al.*, 2005; Crocker *et al.*, 2005; Nishino *et al.*, 2000; Peyron *et al.*, 2000; Ripley *et al.*, 2001; Thannickal *et al.*, 2000). Additionally, researchers have detected a genetic defect in the hypocretin 2 receptor gene in a longstanding dog model of narcolepsy (Lin *et al.*, 1999). Like people with narcolepsy, these dogs experience symptoms of excessive daytime sleepiness and cataplexy. Finally, in a mouse model of narcolepsy, hypocretin ligand and hypocretin receptor knockout (KO) mice, as well as transgenic mice with a degenerative loss of hypocretin neurons, also display a phenotype similar to that of human narcolepsy (Chemelli *et al.*, 1999; Hara *et al.*, 2001; Kisanuki *et al.*, 2000). Interestingly, hypocretin ligand KO mice have also been shown to display deficits in reward/reinforcement processes as well as dopamine signaling indicating reduced responsivity to drugs of abuse (see below). This is particularly notable given that people with narcolepsy rarely develop addiction, despite treatment with psychostimulant drugs such as amphetamine (Akimoto *et al.*, 1960; Hayduk *et al.*, 1995; Rogers *et al.*, 1997; Rogers *et al.*, 2001).

Summary

Both basic and clinical research indicates that the hypocretins participate in the regulation of arousal and arousal-related processes via a widespread projection system that acts upon multiple terminal fields. Given that arousal is a critical factor in the accurate expression of all motivated behaviors, these observations have important implications for potential involvement of hypocretin systems on a broad constellation of physiological functions including those associated with drug reward and reinforcement.

II. Hypocretin regulation of reward and reinforcement processes

An amassing literature indicates that the hypocretin system participates in reward processes and motivated behaviors associated with cocaine. Some of the first evidence to support this emerged from anatomical studies showing prominent hypocretin projections to reward-related brain structures including the VTA and nucleus accumbens (Fadel and Deutch, 2002; Peyron *et al.*, 1998). Additional work also demonstrated that dopamine neurons of the VTA contain both hypocretin 1 and hypocretin 2 receptors and that hypocretins exert excitatory actions on these neurons (Borgland *et al.*, 2006; Korotkova *et al.*, 2003; see below). Given these observations, it is not surprising that multiple reports have focused on the extent to which the hypocretin system influences behavioral and dopamine responses to cocaine and other drugs of abuse.

Drugs of abuse recruit the activity of hypocretin neurons

In a series of studies from multiple research groups, it was demonstrated that experience with drugs of abuse results in the activation of hypocretin-producing neurons. For example, acute injections of methamphetamine or nicotine (Ko *et al.*, 2003; Pasumarthi *et al.*, 2006) increased Fos immunoreactivity in hypocretin neurons to over 300% of vehicle-treatment levels. Additionally, chronic nicotine produced an increase of hypocretin mRNA levels, as well as mRNA levels of both hypocretin 1 and 2 receptors (Kane *et al.*, 2000). Discontinuation of chronic drug delivery and subsequent withdrawal can also elicit activation of hypocretin neurons. Indeed, naloxone-induced withdrawal from chronic morphine treatment increases Fos levels of hypocretin peptide mRNA in hypocretin neurons and also increases Fos in the NAc shell (Georgescu *et al.*, 2003; Sharf *et al.*, 2008; Zhou *et al.*, 2006). Interestingly, it appears that hypocretin 1 receptors are important in these actions given that the hypocretin 1 receptor antagonist, SB-334867, reduces the effects of morphine withdrawal on Fos levels in the NAc shell and attenuates symptoms of morphine withdrawal (Sharf *et al.*, 2008). In addition hypocretin neurons show increased Fos when animals are exposed to cues that once predicted ethanol availability (Dayas *et al.*, 2008).

Conditioned place preference

The previous observations suggest that administration of various drugs of abuse appears to engage hypocretin neuronal activity, however, it is possible that the increases in Fos observed with these drugs may be associated with generalized enhancement of arousal, and not with more direct actions on reward mechanisms. Despite this possibility, two studies suggest that this may not be the case for conditioned place preference (CPP) for cocaine or morphine. In those studies, Harris and colleagues demonstrate that the increases in Fos are observed only in animals that developed CPP for morphine or cocaine but not in those that were treated with drug, yet failed to acquire CPP (Harris and Aston-Jones, 2006; Harris *et al.*, 2005). Thus, rats that acquired cocaine or morphine CPP had 3-fold greater numbers of Fos-positive hypocretin neurons than naïve animals. Animals that were treated with these drugs but did not acquire CPP showed levels of Fos comparable to naïve rats. Interestingly, the degree of Fos activation was also shown to be proportional to the magnitude of CPP induced by chemical activation of hypocretin neurons, further suggesting that the activity of these neurons is associated with the rewarding aspects of these drugs. Consistent with these observations, hypocretin signaling has also been shown to be necessary for reinstatement of CPP. For example, in animals that had been extinguished of morphine CPP behavior, activation of hypocretin neurons or hypocretin-1 injections into the VTA reinstated CPP (Harris *et al.*, 2005).

The importance of hypocretin 1 receptors in reward processes is again highlighted in a series of CPP experiments using SB-334867. Systemic pretreatment with SB-334867 blocks CPP for morphine (Harris *et al.*, 2005) as well as CPP for amphetamine (Hutcheson *et al.*, 2011). Additionally, infusions of SB-334967 into the VTA block morphine CPP (Narita *et al.*, 2006; Sharf *et al.*, 2010), as do unilateral lesions of the lateral hypothalamus combined with contralateral SB-33467 infusions into the VTA (Harris *et al.*, 2007). Surprisingly, while systemic administration of SB-334867 is capable of blocking CPP for morphine (Harris *et al.*, 2005; Sharf *et al.*, 2010) it does not appear to affect CPP for cocaine (Sharf *et al.*, 2010).

As mentioned above, people with narcolepsy, and presumably reduced hypocretin signaling, rarely develop addiction. In this light, it is noteworthy that mice lacking hypocretin peptides show multiple deficits in their responsivity to drugs of abuse. For example, hypocretin KO mice have been shown to be less susceptible than wild-types (WT) in developing morphine dependence (Georgescu *et al.*, 2003), they display reduced locomotor and dopamine responses to morphine, and they do not develop CPP for morphine (Narita *et al.*, 2006; Sharf *et al.*, 2010). Recently, we have shown that hypocretin KO mice are also less sensitive to the rewarding properties of cocaine as these mice fail to exhibit CPP for cocaine (España and Jones, 2010). In those studies, and consistent with an extensive literature, WT mice exhibited robust CPP for cocaine, however, hypocretin KO mice showed a non-significant trend toward conditioned place aversion. When taken together, these observations suggest that hypocretin signaling is important for CPP behavior associated with cocaine and other drugs and thus normal levels of hypocretins may be a critical participant in regulating the rewarding properties of drugs of abuse.

Self-Administration

Multiple paradigms are used to examine drug-seeking and drug-taking in rodents, however, intravenous (i.v.) self-administration of drugs is generally accepted as the gold standard for modeling drug abuse. These techniques can be used to model different aspects of drug taking, including the acquisition and maintenance phases of drug intake as well as the propensity to relapse by measuring reinstatement of operant drug seeking behavior following a period of forced abstinence. Additionally, researchers often vary the schedules of reinforcement to examine specific aspects of cocaine intake, including drug consumption, diurnal variations in cocaine intake, and the motivation to work for cocaine. The section that follows will provide a summary of recent observations indicating a prominent role for the hypocretin system in self-administration of cocaine and other drugs of abuse.

Reinstatement

Reinstatement of drug seeking behavior is a useful method to assess the persistence of lever pressing when the drug is no longer available. Rats are typically trained to self-administer drugs until stable levels of responding are reached and rats are subsequently exposed to extinction sessions. During extinction, rats quickly learn that drug is no longer available and consequently they cease responding on the lever. Once lever responding is extinguished, rats are presented with a cue associated with cocaine intake, or alternatively, they are exposed to a stressor (e.g., foot shock), which leads to reinstatement of drug seeking.

Several observations indicate that hypocretin signaling is crucial for normal reinstatement of drug seeking. For example, i.c.v. infusions of the hypocretin-1 peptide are sufficient to reinstate cocaine and nicotine seeking (Boutrel *et al.*, 2005; Plaza-Zabala *et al.*, 2010). Consistent with this, SB-334967 reduces cue-induced reinstatement of ethanol and cocaine seeking (Lawrence *et al.*, 2006; Smith *et al.*, 2009) and reinstatement of cocaine-seeking following foot shock and yohimbine-induced stress (Boutrel *et al.*, 2005; Richards *et al.*, 2008). Interestingly, cocaine-primed reinstatement is not attenuated with SB-334867 treatment (Smith *et al.*, 2009), although the mechanisms underlying this difference are not clear. When considered together, these observations indicate that the motivation to seek drug

after extinction can be influenced by hypocretin signaling, depending on the parameters used to elicit drug seeking.

Maintenance

The involvement of hypocretins in regulating the reinforcing actions of drugs have been investigated across a battery of self-administration schedules of reinforcement and results have shown varied effects depending on the paradigms used. Initial, observations examining the extent to which the hypocretin system influences ongoing cocaine self-administration suggested that blocking hypocretin signaling had no effect on the maintenance of cocaine intake. In these studies, rats were implanted with a catheter into the right jugular vein and were trained to self-administer cocaine on a fixed ratio 1 (FR1) schedule of reinforcement in which a single lever press resulted in delivery of a relatively large dose of cocaine (Aston-Jones *et al.*, 2009; España *et al.*, 2010). Under these conditions, rats are able to attain preferred blood levels of cocaine with little effort and thus rats can readily maintain those levels throughout a session. Consequently, responding on an FR1 schedule provides information about an animal's preferred level of cocaine consumption. After reaching stable patterns of cocaine intake, rats were pretreated with either vehicle or SB-334867 and cocaine intake was measured. As previously described (Aston-Jones *et al.*, 2009; España *et al.*, 2010), treatment with SB-334867 had no effect on cocaine consumption. A similar lack of effect on an FR1 schedule was also observed when rats were infused with the hypocretin-1 peptide directly into the lateral ventricles (Boutrel *et al.*, 2005; España *et al.*, 2011). Although these data suggested that active cocaine self-administration was not affected by hypocretin signaling, subsequent findings using different schedules of reinforcement with varying availability levels or effort requirements demonstrated significant effects of hypocretin manipulations on self-administration of cocaine and other drugs of abuse.

In a series of studies, we tested the effects of hypocretin manipulations across a battery of self-administration schedules. Using a 24 hr access, discrete trials (DT) schedule of reinforcement, rats were kept from maximizing their blood levels of cocaine by restricting cocaine access to three trials per hour. This degree of cocaine access engenders a characteristic pattern of intake in which animals engage in cocaine intake almost exclusively during the dark-phase (Roberts *et al.*, 2002). Although, the effort requirement is low in a DT paradigm (one lever response results in cocaine delivery), the complex interaction between dose and availability makes this schedule more susceptible to pharmacological and physiological influences than schedules with less restricted access. As shown in figure 1, injections of SB-334867 produce a marked reduction of cocaine intake that occurred within 20 minutes of SB-334867 treatment, particularly in rats treated with the highest dose of SB-334867 (España *et al.*, 2010). In contrast, i.c.v. infusions of hypocretin-1 increased cocaine intake, thereby extending periods of cocaine intake (España *et al.*, 2011). These effects of hypocretin manipulations are similar to other drugs that selectively reduce intake under DT, but not FR1 schedules (Brebner *et al.*, 2000; Smith *et al.*, 2004).

The progressive ratio (PR) schedule of reinforcement is designed to assess an animal's motivation to work for a reinforcer and provides information on changes in the reinforcing

efficacy of drugs. During the early portions of a PR session, single cocaine injections are obtained with relatively low effort (few lever responses), however, as the session continues, the lever response requirement increases and thus rats must exert progressively greater effort to obtain a single cocaine injection. Using the PR schedule, we demonstrated that both i.p. and bilateral intra-VTA injections of SB-334867 reduced the motivation for an animal to work for cocaine. Specifically, animals treated with SB-334867 engaged in significantly less lever pressing and reached lower breakpoints (fewer cocaine injections) than animals treated with vehicle (Fig. 2A). These effects are similar to recent reports showing that a 10 mg/kg i.p. dose of SB-334867 reduces PR responding for cocaine, as well as nicotine and heroin (Borgland *et al.*, 2009; Hollander *et al.*, 2008; Smith and Aston-Jones, 2010). Consistent with these findings, enhancement of hypocretin signaling produces the opposite effect. Thus, as shown in figure 2B, bilateral infusions of the hypocretin-1 peptide directly into the VTA, elicit an increase in breakpoints and the number of lever responses, further indicating the importance of hypocretin signaling in cocaine reinforcement (España *et al.*, 2011).

Another self-administration procedure, termed the threshold schedule of reinforcement, has been used to examine both consumption and motivation within a single session (España *et al.*, 2010). In this procedure, lever responses during the early portion of the session result in high doses of cocaine, similar to that observed with an FR1 schedule. During this portion of the session, rats can readily titrate to preferred blood levels of cocaine, and thus it is not surprising that SB-334867 has no effect on cocaine intake under these conditions (Fig. 3A). However, as the dose of cocaine is lowered across the session, rats are required to respond more frequently to maintain blood levels of cocaine. This is reminiscent of a PR schedule in which increasingly greater numbers of responses are required to obtain the same amount of drug. At this higher price of cocaine, injections of SB-334867 decrease responding earlier in the session, at 'threshold' doses that are sufficient to maintain self-administration in vehicle-treated rats (Fig. 3B).

Summary

Combined, the described self-administration studies indicate that hypocretin neurotransmission is necessary to support cocaine self-administration under schedules of reinforcement that require high-effort or where titrating to preferred blood levels of cocaine is restricted. Furthermore, given that intra-VTA SB-334867 and i.p. SB-334867 reduced cocaine intake to a similar extent, these observations provide strong support for the hypothesis that hypocretin influences cocaine reinforcement, via actions within the VTA.

III. Hypocretin regulation of dopamine release and uptake

A series of studies using behavioral, genetic, electrophysiological, and neurochemical techniques have expanded our understanding of hypocretin involvement in arousal, particularly as it relates to the regulation of motivated behavior, reward/reinforcement processes, and the neural mechanisms underlying these actions. These studies have shown convincingly that enhancement of hypocretin neurotransmission promotes behavioral and neurochemical responses to drugs of abuse, while manipulations that disrupt hypocretin signaling attenuate these responses. Given that the focus of the present review involves hypocretin regulation of dopamine function and motivated behaviors reliant on dopamine

signaling, it is helpful to briefly review the importance of the mesolimbic dopamine system in the regulation of reward and reinforcement processes associated with cocaine.

Dopamine involvement in reward and reinforcement mechanisms

An extensive literature has demonstrated the importance of the mesolimbic projections from the VTA to the ventral striatum in reward and reinforcement processes associated with cocaine and other drugs of abuse (Koob and Le Moal, 1997; Wise, 1996; Woolverton and Johnson, 1992). Cocaine inhibits re-uptake of dopamine into the presynaptic terminal by blocking the dopamine transporter (DAT) and through these actions increases extracellular levels of dopamine and activation of pre- and post-synaptic dopamine receptors in VTA target regions (Fig. 4). It is generally believed that this enhancement of dopamine is involved in cocaine's rewarding and reinforcing effects.

Pharmacological manipulations were among the first to suggest that enhanced dopamine signaling produced rewarding/reinforcing effects while attenuation of dopamine signaling leads to reduced reinforced behavior. For instance, various dopamine agonists produce CPP (Hoffman and Beninger, 1988; Hoffman and Beninger, 1989; Hoffman *et al.*, 1988; Papp, 1988; Parker, 1992; White *et al.*, 1991), are readily self-administered (Caine *et al.*, 1999; Self *et al.*, 1996; Self and Stein, 1992; Weed and Woolverton, 1995), and have cocaine-like discriminative stimulus effects (Sinnott *et al.*, 1999; Sinnott and Nader, 2001; Spealman *et al.*, 1991). In contrast, dopamine antagonists can produce conditioned place aversion, (Acquas and Di Chiara, 1994; Cervo and Samanin, 1995; Funada and Shippenberg, 1996; Hoffman and Beninger, 1988; Hoffman and Beninger, 1989; Shippenberg *et al.*, 1991; Shippenberg and Herz, 1987; White *et al.*, 1991), and attenuate cocaine self-administration (Caine and Koob, 1994).

Among the multiple limbic structures involved in dopamine regulation of reward and reinforcement, the NAc stands out as critical participant in psychostimulant self-administration. The vast majority of addictive drugs increase extracellular dopamine in the NAc (Carboni *et al.*, 1989; Di Chiara and Imperato, 1988; Imperato and Di Chiara, 1986) and NAc neurons display discrete discharge activity in response to cocaine-reinforced responding during self-administration (Carelli *et al.*, 1993; Uzwiak *et al.*, 1997). Additionally, lesions and pharmacological suppression of dopamine signaling within the NAc indicate that cocaine self-administration requires an intact NAc (Lyness *et al.*, 1979; McGregor and Roberts, 1995; Roberts *et al.*, 1977). These observations, amongst many others, support the hypothesis that the mesolimbic dopamine system, including the connection from the VTA to the NAc, is an essential participant in the reinforcing and behavioral effects of cocaine.

Hypocretin neurotransmission modulates dopamine responses to cocaine

As mentioned above, hypocretin neurons send relatively strong projections to the VTA where hypocretin fibers make synaptic contacts with dopamine neurons (Fadel and Deutch, 2002; Peyron *et al.*, 1998). Additionally, both hypocretin-1 and -2 peptides induce burst firing of dopamine neurons and potentiate their glutamatergic-mediated excitation (Borgland *et al.*, 2006; Korotkova *et al.*, 2003) indicating that the hypocretin neurons are poised to

regulate dopaminergic function. Given these observations, and the general involvement of dopamine systems in regulating motivated behaviors associated with drugs of abuse, it is likely that hypocretin influences reinforcement processing via actions on mesolimbic dopamine systems.

Some of the first evidence that hypocretin systems participated in regulating dopamine responses to drugs of abuse came from studies investigating the pharmacological effects of hypocretin agents on dopamine signaling. Under baseline conditions, infusions of hypocretin-1 into the VTA were shown to increase extracellular levels of dopamine in the prefrontal cortex and NAc of rats (Narita *et al.*, 2006; Vittoz and Berridge, 2006; Vittoz *et al.*, 2008). Consistent with this, hypocretin KO mice show reduced dopamine responses to morphine, despite showing normal levels of extracellular dopamine under baseline conditions (Narita *et al.*, 2006). In recent microdialysis and voltammetry studies we have also shown that hypocretins regulate dopamine signaling, particularly in response to cocaine. In the first set of studies, rats were implanted with microdialysis probes to measure extracellular dopamine levels in the NAc core. On testing days, rats were pretreated with i.p. vehicle or 30 mg/kg SB-334867, forty minutes prior to receiving a single 10 mg/kg i.p. injection of cocaine. In rats treated with vehicle, injections of cocaine produced robust increases in extracellular dopamine levels (Fig. 5A). In contrast, the effects of cocaine in animals treated with SB-334867 were attenuated to nearly half of what was observed in vehicle controls (España *et al.*, 2010). In another set of experiments, animals were implanted with probes in the NAc and also with bilateral infusion cannulae in the VTA. Rats were pretreated with vehicle or SB-334867 (10 nmol) directly into the VTA and as before received a single i.p. injection of cocaine. Similar to that observed with i.p. injections, SB-334867 reduced the effects of cocaine on dopamine signaling. Interestingly, unlike that seen with i.p. delivery, SB-334867 into VTA also reduced baseline levels of dopamine prior to cocaine administration.

A similar set of results was also obtained in studies using voltammetry in anesthetized rats. Animals were implanted with an i.v. jugular catheter, a recording electrode in the NAc core and an infusion cannula/stimulating electrode in the ipsilateral VTA. Following collection of a stable baseline of electrically-stimulated dopamine release, rats received an infusion of vehicle or SB-334867 (10 nmol) directly into the VTA. Forty minutes later, rats received a single i.v. injection (1.5 mg/kg) of cocaine and changes in dopamine release and uptake were measured. When compared to vehicle-treated animals, SB-334867 produced a significant reduction in electrically stimulated dopamine release prior to cocaine injection, further indicating that hypocretin signaling regulates dopamine neurotransmission (Fig. 5B and C). In addition to these effects, SB-334867 also reduced the effects of cocaine on dopamine signaling. Relative to vehicle treatment, SB-334867 reduced the effects of cocaine on both stimulated dopamine release and dopamine uptake inhibition.

In another set of experiments, we used *in vitro* voltammetry in brain slices from WT and hypocretin KO mice to examine whether a complete loss of hypocretin signaling would also produce deficits in dopamine signaling. Recording and stimulating electrodes were positioned within the NAc core to allow for measurement of locally-evoked dopamine release. Following a stable baseline of dopamine release, cocaine was bath-applied to the

slice at cumulatively higher concentrations. Under baseline conditions, disruption of hypocretin neurotransmission in KO mice results in disrupted dopamine signaling, with a reduction in both evoked dopamine release and dopamine uptake rate (Fig. 6). Furthermore, hypocretin KO mice displayed attenuated dopamine responses to cocaine, showing only half as much dopamine uptake inhibition as WT mice (España *et al.*, 2010).

Given that disruptions in hypocretin signaling reduce dopamine responses to cocaine, we next examined whether enhancement of hypocretin signaling would have the opposite effect. Initial experiments used microdialysis to measure extracellular levels of dopamine in the NAc core. Experiments were conducted as described above with the exception that all animals received vehicle or hypocretin-1 (0.5 nmol) directly into the VTA, 20 mins prior to receiving a 10 mg/kg cocaine injection. As shown in figure 7A, infusions of hypocretin-1 had no effect on dopamine levels under baseline conditions (España *et al.*, 2011). Nevertheless, when compared to vehicle, hypocretin-1 significantly augmented the effects of cocaine at increasing dopamine levels. Voltammetry studies in anesthetized rats also showed a hypocretin enhancement of cocaine's effects. In those experiments, rats were pretreated with vehicle or hypocretin-1 (0.5 nmol) into the VTA 20 min prior to receiving an i.v. injection of 1.5 mg/kg cocaine (España *et al.*, 2011). Within 5 min of infusion, hypocretin-1 significantly increased evoked dopamine release prior to cocaine treatment. Further, hypocretin-1 infusions also augmented the effects of cocaine on evoked dopamine release and dopamine uptake inhibition (Fig. 7B and C).

Summary

The neurochemical studies described above provide compelling evidence for the hypothesis that hypocretin neurotransmission influences dopamine signaling in the NAc core, particularly in response to cocaine. Moreover, given that many of the described experiments used intratissue infusions of agents into the VTA, these studies indicate that the actions of hypocretin on dopamine release and uptake involve signaling within the VTA.

IV. Conclusions

The hypocretin projection to dopamine neurons of the VTA appears to be a critical modulator of reward and reinforcement processing and dopamine signaling in the ventral striatum. As described above, amassing evidence indicates that an intact hypocretin system is necessary for normal behavioral responses to various drugs of abuse, including cocaine. Studies using multiple self-administration schedules of reinforcement demonstrate clearly that disruption of hypocretin signaling reduces the motivation for animals to take cocaine under conditions that require effortful responding or in which access to cocaine is limited. Consistent with these observations, enhancement of hypocretin signaling exerts the opposite influence by increasing the motivation to take cocaine.

The actions of hypocretin on reward and reinforcement behavior are likely to be associated with modulation of dopamine signaling within the striatum. Neurochemical and electrophysiological studies show convincingly that manipulations to hypocretin neurotransmission result in disrupted dopamine signaling that lead to altered dopamine levels within the NAc core both under baseline conditions and in responses to cocaine and

other drugs of abuse. When taken together, both behavioral, electrophysiological, and neurochemical studies provide significant evidence for the hypothesis that the hypocretin system participates in reward and reinforcement processes, particularly as it relates to cocaine, via actions on the mesolimbic system. Further these observations suggest that continued research in this area of drug addiction may lead to development of rational pharmacotherapies based on hypocretin signaling.

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Figure 1. Hypocretin signaling regulates cocaine self-administration on a DT schedule of reinforcement

(A) Shown is a response pattern from an individual rat that received an i.p. vehicle (white arrow) or 30 mg/kg SB-334867 injection (black arrow). Horizontal rasters represent 24 hr periods. Vertical tick marks represent trials in which a 1.5 mg/kg cocaine injection was taken. (B) Shown are the mean $\pm$ SEM number of cocaine injections taken over the 6-hr period following i.p. injections of vehicle (n=8) or SB-334867 (7.5, 15 and 30 mg/kg; n=8). (C) Shown is a response pattern from an individual rat that received a vehicle (white arrow) or 0.5 nmol hypocretin-1 infusion (black arrow) into the lateral ventricle. (D) Shown are the

mean $\pm$ SEM number of cocaine injections taken over the 6 hr period following infusion of vehicle (n=7) or 0.5 nmol hypocretin-1 (n=7). Note that for SB-334867 experiments, rats were treated at 11:00 am when rats are typically awake, whereas for hypocretin-1 experiments, rats were treated at 3:00 pm a time when rats typically discontinue taking cocaine. * $P < 0.05$, ** $P < 0.01$ relative to baseline.

Figure 2. Hypocretin signaling within the VTA regulates cocaine self-administration on a PR schedule of reinforcement

(A) Shown are the mean $\pm$ SEM number of cocaine breakpoints and lever responses following unilateral or bilateral intra-VTA infusions of vehicle (unilateral, n=5; bilateral, n=6) or 10 nmol SB-334867 (uni, n=5; bilat, n=6). (B) Shown are the mean $\pm$ SEM number of cocaine breakpoints and lever responses following unilateral or bilateral intra-VTA infusions of vehicle (uni, n=5; bilat, n=6) or 0.5 nmol hypocretin-1 (unilateral, n=5; bilateral, n=6) * $P < 0.05$, ** $P < 0.01$ relative to baseline.

Figure 3. SB-334867 reduces responding as the unit price of cocaine is increased

(A) Shown are the mean $\pm$ SEM percent baseline consumption of cocaine following i.p. injection of vehicle (n=9) or SB-334867 (7.5, 15 or 30 mg/kg n=9) on the threshold schedule of reinforcement. (B) Shown are the mean $\pm$ SEM P_{max} values, expressed as a percentage of baseline, following i.p. injections of vehicle or SB-334867. The maximal unit price, P_{max} , is defined as the point at which maximal responding occurs before an animal discontinues taking cocaine and is a measure of the behavioral economic index of price. * $P < 0.05$, ** $P < 0.01$ relative to vehicle.

Figure 4. Effects of cocaine at the dopamine transporter

(Left) Under normal conditions, dopamine (DA) neuron firing results in dopamine-filled vesicles to fuse with the presynaptic membrane and subsequently release into the synaptic cleft. Once in the synapse dopamine can bind to post-synaptic dopamine receptors and then is removed from extracellular space by the dopamine transporter (DAT). Once dopamine is back inside the presynaptic terminal, it is repackaged into synaptic vesicles for future release via the vesicular monoamine transporter (VMAT). (Right) Cocaine increases extracellular levels of dopamine by interacting with the DAT and thereby inhibiting dopamine uptake back into the terminal. As dopamine uptake is reduced, levels accumulate in the synapse and dopamine has a greater opportunity to bind to dopamine receptors.

Figure 5. SB-334867 attenuates cocaine-induced elevations in dopamine signaling in the NAc core

(A) Shown are the mean $\pm$ SEM of extracellular levels of dopamine (DA) within the NAc core following i.p. injection of vehicle ($n=6$) or 30 mg/kg SB-334867 ($n=6$). Shown are representative concentration-time plots and cyclic voltammograms (insets) of dopamine responses from rats that received pretreatment infusions of (B) vehicle or (C) 10 nmol SB-334867 into the VTA. Stim represents the time of electrical stimulation (1 sec, 60 Hz pulse). (Insets in B and C) Cyclic voltammograms depict two current peaks, one at 600 mV (positive deflection) for dopamine oxidation and one at -200 mV (negative deflection) for reduction of dopamine-o-quinone. The position of the peaks identifies the substance oxidized as DA. * $P<0.05$, ** $P<0.01$ relative to vehicle.

Figure 6. Hypocretin knockout mice display disrupted baseline dopamine signaling and attenuated dopamine responses to cocaine

Shown are the mean $\pm$ SEM of (A) stimulated dopamine (DA) release and (B) maximal dopamine uptake rate ($V_{\max}$) in WT ($n=7$) and hypocretin KO ($n=7$) mouse slices containing the NAc core. (C) Shown are the mean $\pm$ SEM DA uptake inhibition (apparent K_m) in WT ($n=7$) and hypocretin KO ($n=7$) mouse slices across cumulative doses of cocaine. * $P<0.05$, ** $P<0.01$ relative to vehicle.

Figure 7. Hypocretin-1 augments cocaine-induced elevations in dopamine signaling in the NAc core

(A) Shown are the mean $\pm$ SEM of extracellular levels of dopamine (DA) within the NAc core following intra-VTA infusion of vehicle ($n=6$) or 0.5 nmol hypocretin-1 (HCRT-1; $n=6$). Shown are representative concentration-time plots and cyclic voltammograms (insets) of dopamine responses from rats that received pretreatment infusions of (B) vehicle or (C) 0.5 nmol hypocretin-1 into the VTA. Stim represents the time of electrical stimulation (1 sec, 60 Hz pulse). (Insets in B and C) Cyclic voltammograms depict two current peaks, one at 600 mV (positive deflection) for dopamine oxidation and one at -200 mV (negative deflection) for reduction of dopamine-o-quinone. The position of the peaks identifies the substance oxidized as DA. * $P<0.05$, ** $P<0.01$ relative to vehicle.