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Differential orexin/hypocretin expression in addiction-prone and -resistant rats selectively bred for high (HiS) and low (LoS) saccharin intake

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Abstract

Rats that have been selectively bred for high (HiS) saccharin intake demonstrate elevated drug-seeking behavior in several phases of addiction compared to those bred for low (LoS) saccharin intake. HiS rats also consume greater amounts of highly palatable substances compared to LoS rats; however, little is known about the neurobiological substrates moderating the divergent behaviors found between the HiS and LoS lines. Orexins are neuropeptides that have been implicated in the conditioned cue aspects of drug abuse and overconsumption of palatable substances, and differential orexin activity in the HiS and LoS phenotypes may enhance our understanding of the close relationship between food and drug reward, and ultimately food and drug addiction. The lateral hypothalamus (LH) and perifornical area (PFA) are brain regions that have been implicated in regulating feeding behavior and addiction processes, and contain orexinergic neurons that project broadly throughout the brain. Thus, we investigated orexin and c-Fos expression in the LH and PFA using immunohistochemistry in HiS and LoS rats following either control or cocaine (15 mg/kg) injections. Results indicated that HiS rats have higher orexin-positive cell counts compared to LoS rats in both the LH and PFA, regardless of cocaine (vs. saline) treatment. In contrast, neuronal activity indicated by c-Fos expression did not differ in either of these brain areas in HiS vs. LoS rats. These results suggest that the orexin system may be involved in aspects of genetically mediated differences in vulnerability to compulsive, reward-driven behaviors.

Keywords

addiction; cocaine; immunohistochemistry; orexin; saccharin preference; selective breeding

Introduction

Rats that have been selectively bred for high (HiS) or low (LoS) saccharin intake display drug-prone and -resistant profiles, respectively [1]. For instance, HiS (vs. LoS) rats consume more ethanol [5], escalate cocaine self-administration at faster rates, and exhibit greater reinstatement of cocaine-seeking behavior in an animal model of relapse [16]. In addition to divergent drug-related responses, HiS rats also have greater avidity than LoS rats for foods rich in fat and sugar [9, 26], as well as liquids sweetened with a variety of natural and

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artificial substances [4, 6]. While these studies provide a behavioral association between genetically-mediated differences in response to drugs of abuse and non-drug rewards, little is known about the neurobiological substrates underlying these differences.

One plausible mechanism involves the orexins (also called hypocretins). Two types of these neuropeptide hormones have been identified (orexin-A and -B), and both originate from cell groups segregated to the lateral hypothalamus (LH) and perifornical area (PFA) that project to brain regions associated with reward processing [17]. Two types of orexin receptors (type 1 and 2) have also been identified, with the type-1 receptor showing greater affinity for orexin-A and the type-2 showing similar affinity for both orexin-A and -B [19]. Activity of the orexin type-2 receptor has been implicated in arousal and sleep regulation [25], while the orexin type-1 receptor is involved in the association between rewards and contextual stimuli [22]. Importantly, orexin has been shown to mediate behaviors related to both drug and non-drug rewards [2, 24]. For example, orexin-knockout mice consume less sucrose solution compared to wild-type mice [13], and mRNA levels of orexin are increased following intake of a saccharin solution [8] and binge patterns of sugar consumption [15]. Additional research has suggested that orexin administration facilitates cue-induced overconsumption of highly palatable foods [3], while orexin type-1 receptor antagonism reduces feeding behavior [18]. Additionally, antagonism of this receptor attenuates ethanol consumption, reinstatement of cocaine- and ethanol-seeking behavior [12, 20], and motivation for cocaine as assessed by a progressive-ratio schedule of reinforcement in rodents [7], suggesting that the orexinergic system is involved not only in feeding behavior, but in the addiction process as well.

Given the apparent relationship of orexin in the behavioral responses that distinguish the HiS and LoS phenotypes, the primary aim of the present study was to compare orexin expression in the LH and PFA between these lines using immunohistochemical procedures. These areas were chosen because the orexinergic projections found in brain regions associated with reward processing originate from cell groups isolated in the LH and PFA [18]. A second aim of this study was to examine relative differences in the activation of orexin neurons measured by c-Fos expression in these same brain areas following acute cocaine exposure in the HiS and LoS rats.

Materials and methods

Twelve HiS and 12 LoS female rats approximately 90–120 days old served as subjects in this experiment. These rat lines were derived from a selective breeding program originating from Occidental College (Los Angeles, CA) and maintained at the University of Minnesota (Minneapolis, MN) using procedures described elsewhere [5]. Briefly, rats are bred based on extremely high or low intake of saccharin solution compared to baseline water intake, resulting in high and low saccharin-preferring phenotypes that have remained stable in a multitude of generations over 10 years. Rats were bred and pair-housed in plastic cages with ad libitum access to rat chow (Purina Mills, Minneapolis, MN, USA) and water prior to the experiment. Humidity, temperature (21–23°C), and light-dark cycle (12 h–12 h; lights on at 6:00 a.m.) were all regulated. All procedures followed guidelines established by the National Research Council and were approved by the University of Minnesota Institutional Animal Care and Use Committee under protocol # 1008A87754. Animal housing and laboratory facilities were accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC).

Six rats from both HiS and LoS phenotypes were randomly selected to receive a single cocaine injection (15 mg/kg, i.p.), while 6 rats from both phenotypes received saline (0.9% sterile NaCl, equivalent volume, i.p.). Cocaine was provided by National Institute of Drug

Abuse (Research Triangle Institute, Research Triangle Park, NC). This dose of cocaine was chosen because it has been shown to increase c-Fos expression in multiple brain areas associated with reward without being so large that phenotypic differences would be obscured by ceiling effects [23]. One and a half hours following injections, rats were deeply anesthetized with Sleepaway (0.5–0.65 ml/animal, i.p., Fort Dodge Laboratories, Fort Dodge, IA). Next, the animals were perfused transcardially, and tissue was processed in an identical manner to that described elsewhere [10]. Briefly, multiple coronal slices (40 μ m) were taken at the level of the LH/PFA and first labeled for c-Fos (sc-52, Santa Cruz Biotechnologies, Inc, Santa Cruz, CA; 1:3000). The slices were incubated in biotinylated secondary (Vector Laboratories, Burlingame, CA; 1:200) and then 0.08% diaminobenzidine tetrahydrochloride (DAB) (Sigma-Aldrich Chemicals, St. Louis, MO) with nickel chloride (0.03%) added to intensify staining. Tissue samples were then labeled for orexin (orexin A antibody, Santa Cruz Biotechnology, Santa Cruz, CA; 1:1,000; biotinylated secondary, Vector Laboratories, Burlingame, CA; 1:500), incubated in DAB (without nickel chloride), mounted on slides, and cover-slipped. Slides were photographed, and stained neurons were counted from two slices per animal. For each animal, the two slices that had the most stained cells were chosen, and these slices were not always contiguous. Double labeling produced discernable staining between orexin (large, irregularly shaped cell bodies) and c-Fos (small, circular nuclei). Photographs were oriented such that the fornix was centered with approximately 200 μ m above and 200 μ m below its dorsal and ventral edges, respectively. Labeled cells up to 300 μ m medial to the lateral-most edge of the fornix were considered to be in the PFA, while cells up to 250 μ m lateral to the fornix were considered to be in the LH (see Fig. 1, panel A). Cells were counted and categorized as expressing c-Fos only (c-Fos+/orexin-), orexin only (c-Fos-/orexin+), or both c-Fos and orexin (c-Fos+/orexin+) (see Fig 1, panel B). Cell counts were averaged between the two slices per animal and verified by an observer who was blind to the experimental groups.

A total of 6 2-factor ANOVAs were conducted, in which the factors were treatment (cocaine vs. saline) and phenotype (HiS vs. LoS). These individual analyses compared total orexin-labeled neurons (e.g., both c-Fos-/orexin+ and c-Fos+/orexin+), c-Fos+/orexin+ cells only, and c-Fos+/orexin- cells only in either the LH or PFA. Cell counts were also compared in the LH and PFA as percent of orexin+ cells that were also c-Fos+ using a 2-factor ANOVA. Post-hoc comparisons were made using the Tukey- Kramer procedure, and results were considered significant if $p < 0.05$.

Results

For total orexin-labeled neuron counts, there was a main effect of phenotype (HiS > LoS) in both the LH ($F_{1,20} = 7.18$, $p < 0.05$) (Fig. 2, panel A) and PFA ($F_{1,20} = 8.34$, $p < 0.01$) (Fig. 2, panel B). There was also a main effect of phenotype for the c-Fos+/orexin+ cell counts (HiS > LoS) in the LH ($F_{1,20} = 7.26$, $p < 0.05$) (Fig. 3, panel A) and PFA ($F_{1,20} = 8.85$, $p < 0.01$) (Fig. 3, panel B). There were no significant differences between groups in % c-Fos+/orexin+ cells in the LH (mean ($\pm$ SEM) HiS-saline = 36.32 ($\pm$ 3.89); LoS-saline = 38.59 ($\pm$ 2.88); HiS-cocaine = 45.05 ($\pm$ 6.04); LoS-cocaine = 38.38 ($\pm$ 10.56)) or PFA (mean ($\pm$ SEM) HiS-saline = 50.55 ($\pm$ 4.27); LoS-saline = 37.56 ($\pm$ 3.83); HiS-cocaine = 54.55 ($\pm$ 6.75); LoS-cocaine = 48.29 ($\pm$ 6.74)). There were no main effects for treatment or interaction effects following any of these comparisons. Furthermore, there were no differences found when comparing c-Fos+/orexin- cells in either area (data not shown), nor were differences found following post hoc procedures for any analysis mentioned. Thus, total orexin-labeled cell counts were higher in HiS rats compared to LoS rats in both the LH and PFA; however, cocaine treatment had no effect on c-Fos or orexin immunoreactivity in these regions in either phenotype.

Discussion

As orexin-labeled cells must express minimum intracellular orexin levels to reach detection thresholds, one interpretation of these results is that HiS rats produce greater tonic levels of orexin compared to LoS rats. Alternatively, the HiS rats may simply have more orexinergic cells in the LH and PFA compared to the LoS rats. While further research is necessary to determine which interpretation is correct, growing literature illustrating the role of orexin in reward-based behaviors suggests that this relative difference in orexin may contribute to the behavioral differences observed between the HiS and LoS lines [2,24]. Since orexin-expressing cells in the LH and PFA project broadly throughout the brain, there are many possible neurobiological mechanisms by which differential orexin expression may mediate behavior.

One possibility involves the influence of orexin receptors in the nucleus accumbens, a key brain structure involved in reward processing. For example, España et al. [7] showed that administration of an orexin antagonist attenuated cocaine-induced increases of dopamine transmission in the nucleus accumbens and reduced cocaine and sucrose self-administration. Thus, genetically mediated differences in the orexin system may engender variation in reward processing via the nucleus accumbens, resulting in heterogeneous vulnerability to maladaptive behaviors such as compulsive eating and drug addiction. In this regard, the relatively hyperactive orexin system in the HiS rats may elevate vulnerability to compulsive behaviors by enhancing the rewarding aspects of drug taking or overconsumption of palatable foods.

Regardless of the mechanisms involved, these results suggest that variance in reward-related behaviors may be mediated, in part, by genetically determined variations in the orexin system. A study by Moorman and Aston-Jones [14] supported this notion by showing that an orexin receptor antagonist was more effective at reducing ethanol consumption in outbred rats selected for high (vs. low) ethanol consumption. Similar to the HiS/LoS lines, outbred rats selected for high ethanol consumption ingested more saccharin than rats selected for low ethanol consumption [21]. In addition to providing another line of evidence implicating orexin in the heterogeneity of addiction vulnerability, these data suggest that, while the orexin system may be a potential mechanism for pharmacological interventions, such effects may be moderated by phenotype. Recently, we have shown that drugs targeting the GABAergic system are more effective at reducing drug self-administration in LoS rats compared to HiS rats [11]. Future research may further investigate the neurobiological mechanisms involved in mediating phenotypic sensitivity to different pharmacological approaches, such as those targeting the orexin or GABA systems.

The present study represents an initial step in evaluating neurobiological differences between the HiS and LoS lines in general and the orexin system in particular. As such, the finding that acute cocaine administration had no effect on expression of orexin or c-Fos in the LH or PFA was not entirely unexpected, and supports the concept that orexin primarily acts to facilitate associations between contextual stimuli and rewards [2]. In this respect, the single acute dose of cocaine in the present experiment may not be sufficient to capture differences elicited by contextual cues. Future experiments may investigate more cocaine doses or look more directly at the how differential tonic orexin production interacts with contexts of chronic drug and non-drug reward exposure to produce the distinct HiS and LoS behavioral phenotypes. Of particular interest may be the involvement of orexin on both the rewarding and aversive effects of drug-taking or ingestion of palatable substances. Furthermore, recent research has shown sex differences in orexin activity [27]. The present study lacked the statistical power to investigate estrous cycle phase between the groups, and fluctuations in female gonadal hormones between subjects could account for the lack of

cocaine treatment effects. Estrous cycle phase was not monitored in the present study; however, animals were randomly assigned to treatment groups and therefore estrous cycle was assumed to be represented equally between groups. Future research may look more directly at the interaction of cycle phase and phenotypic differences in drug-induced orexinergic neuron activation.

In conclusion, HiS rats exhibited greater orexin production in the LH and PFA compared to LoS rats. These results support recent literature illustrating the role of the orexin system in maladaptive behaviors such as addiction and overconsumption of high-fat, high-sugar foods and liquids. Furthermore, the present study implicates variation in the orexin system in the heterogeneity of vulnerability to compulsive, reward-driven behaviors observed in the human population. Such variation may be an important consideration when developing pharmacological interventions involving the orexin system.

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- Rats bred for high and low saccharin intake show differential addiction vulnerability
- The orexin neuropeptides are implicated in drug addiction and compulsive eating
- Addiction-prone HiS rats express more orexin-positive cells compared to LoS rats
- Orexin may be involved in phenotypic variation in addiction and compulsive eating

Fig. 1.

Panel A shows a tissue sample of the lateral hypothalamus (LH) and perifornical area (PFA). Cells lateral to the fornix (f) were considered to be in the LH and cells medial to the lateral-most edge of the fornix were considered to be in the PFA. Panel B depicts cells labeled for c-Fos (circular nucleus, marked by arrowhead), orexin only (irregularly shaped cell body with unstained nucleus, marked by truncated arrow), or both (stained cell body and nucleus, marked with elongated arrow).

Fig. 2.

Mean ($\pm$ SEM) orexin-positive cell counts in the lateral hypothalamus (panel A) and perifornical area (panel B) following either cocaine (15 mg/kg; Coc) or saline (Sal) in rats selectively bred for high (HiS) or low (LoS) saccharin intake. The * indicates a main effect of phenotype ($p < 0.05$), such that samples from HiS rats showed greater orexin-positive cells compared to LoS rats. The ** indicates a main effect of phenotype ($p < 0.01$), such that samples from HiS rats showed greater orexin-positive cells compared to LoS rats.

Fig. 3.

Mean ($\pm$ SEM) counts of cells with both orexin- and c-Fos-positive staining in the lateral hypothalamus (panel A) and perifornical area (panel B) following either cocaine (15 mg/kg; Coc) or saline (Sal) in rats selectively bred for high (HiS) or low (LoS) saccharin intake. The * indicates a main effect of phenotype ($p < 0.05$), such that samples from HiS rats showed greater orexin- and c-Fos-positive cells compared to LoS rats. The ** indicates a main effect of phenotype ($p < 0.01$), such that samples from HiS rats showed greater orexin-positive cells compared to LoS rats.