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Lesions of orexin neurons block conditioned place preference for sexual behavior in male rats

Andrea R. Di Sebastiano [a], Hilary E. Wilson-Pérez [b], Michael N. Lehman [a], Lique M. Coolen [a,c,*]

[a] Departments of Anatomy and Cell Biology, University of Western Ontario, London, Ontario, Canada
[b] Neuroscience Graduate Program, University of Cincinnati, Cincinnati, OH, USA
[c] Physiology & Pharmacology, University of Western Ontario, London, Ontario, Canada

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ABSTRACT

The hypothalamic neuropeptide orexin (hypocretin) mediates reward related to drugs of abuse and food intake. However, a role for orexin in sexual reward has yet to be investigated. Orexin neurons are activated by sexual behavior, but endogenous orexin does not appear to be essential for sexual performance and motivation in male rats. Therefore, the goal of the current study was to test the hypothesis that orexin is critically involved in processing of sexual reward in male rats. First, it was demonstrated following exposure to conditioned contextual cues associated with sexual behavior in a conditioned place preference paradigm that cFos expression is induced in orexin neurons, indicating activation of orexin neurons by cues predicting sexual reward. Next, orexin-cell specific lesions were utilized to determine the functional role of orexin in sexual reward processing. Hypothalami of adult male rats were infused with orexin-B-conjugated saporin, resulting in greater than 80% loss of orexin neurons in the perifornical–dorsomedial and lateral hypothalamus. Orexin lesions did not affect expression of sexual behavior, but prevented formation of conditioned place preference for a sexual behavior paired chamber. In contrast, intact sham-treated males or males with partial lesions developed a conditioned place preference for mating. Orexin lesioned males maintained the ability to form a conditioned place aversion to lithium chloride-induced visceral illness, indicating that orexin lesions did not disrupt associative contextual memory. Overall, these findings suggest that orexin is not essential for sexual performance or motivation, but is critical for reward processing and conditioned cue-induced seeking of sexual behavior.

Introduction

The hypothalamic neuropeptide orexin (hypocretin) is found exclusively in the lateral hypothalamic area (LHA) and the perifornical–dorsomedial hypothalamus (PFA-DMH) and consists of two active peptides, orexin-A and orexin-B (de Lecea et al., 1998; Sakurai et al., 1998). Orexin is critical for food intake (de Lecea et al., 1998; Sakurai et al., 1998; Sakurai, 2006; Benoit et al., 2008), arousal and sleep (Chemelli et al., 1999; Lin et al., 1999; Sakurai, 2007a, b; Carter et al., 2009). Recent studies have shown that orexin also plays a critical role in mediation of reward (DiLeone et al., 2003; Aston-Jones et al., 2009; Aston-Jones et al., 2010) and orexin cells have extensive projections throughout the brain, including to reward associated brain areas such as the nucleus accumbens (NAc) and ventral tegmental area (VTA) (Peyron et al., 1998; Fadel and Deutch, 2002; Martin et al., 2002).

Orexin neurons are activated by conditioned contextual cues associated with food and drug reward in a conditioned place preference (CPP) paradigm (de Lecea et al., 2006), a standard paradigm used to determine reward seeking behavior (Tzschentke, 2007). Moreover, excitotoxic lesions of orexin neurons in the LHA or orexin receptor-1 antagonists in the VTA significantly reduce morphine preference in a CPP paradigm (Harris et al., 2007). In addition, LHA orexin neuronal stimulation, and intra-VTA orexin-A administration reinstate morphine CPP following extinction (Harris et al., 2005). Orexin-A administration into the LHA increases self-administration of palatable food (Thorpe et al., 2005) while orexin receptor-1 antagonists block self-administration of food (Nair et al., 2008), ethanol (Lawrence et al., 2006) and nicotine (Hollander et al., 2008). Thus, there is ample evidence that orexin plays a role in reward processing related to food intake and drugs of abuse.

Orexin neurons are activated by sexual behavior in male rats (Muschamp et al., 2007; Di Sebastiano et al., 2010). In addition, exogenous orexin-A administration into the medial preoptic area enhances copulatory performance in male rates, evidenced by shortened latencies to mount and intromission, and increased frequency of mounts and intromissions (Gulia et al., 2003). However, a critical role for endogenous orexin in sexual behavior is not supported by findings that orexin cell-specific lesions do not disrupt

* Corresponding author. University of Western Ontario, Room 460, Medical Sciences Building, London, ON, Canada N6A 3K7.
E-mail address: lique.coolen@schulich.uwo.ca (L.M. Coolen).

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sexual motivation or performance (Di Sebastiano et al., 2010), that intracerebroventricular (ICV) administration of an orexin receptor-1 antagonist does not disrupt sexual motivation (Bai et al., 2009), and that systemic administration of antagonist only slightly inhibits sexual performance (Muschamp et al., 2007). However, a role for endogenous orexin in mediation of sexual reward has yet to be elucidated. Therefore, the goal of the current study was to test the hypothesis that orexin plays a critical role in processing of sexual reward. First, it was determined whether orexin neurons are activated by conditioned cues predicting sexual reward by exposing male rats to an environment associated with prior sexual behavior. Next, orexin cell-specific lesions were utilized to determine a specific role for orexin in sexual reward processing using a CPP paradigm (Agmo and Gomez, 1993; Tenk et al., 2009).

Materials and methods

Animals

Adult male Sprague–Dawley rats (200–250 g) were obtained from Harlan (Indianapolis, IN) or Charles River Laboratories (Sherbrooke, Quebec, Canada) and were pair housed for the duration of experiments in Plexiglas cages. The colony room was maintained on a 12/12 reversed light-dark cycle (lights off at 10 am) with food and water available at all times except during behavioral testing. Female Sprague-Dawley rats were obtained from Harlan (Indianapolis, IN) or Charles River Laboratories (Sherbrooke, Quebec, Canada), were bilaterally ovariectomized and received subcutaneous implants of 5% 17-β-estradiol benzoate in silastic capsules. Sexual receptivity was induced by subcutaneous progesterone injections (500 μg in 0.1 mL of sesame oil) approximately 4 h prior to mating sessions. All procedures were approved by the Animal Care Committees at the University of Cincinnati and the University of Western Ontario and conform to guidelines outlined by the National Institute of Health and the Canadian Council on Animal Care.

Experimental design

Experiment 1: cFos expression studies

Apparatus. The CPP apparatus (MED Associates, St. Albans, VT) consisted of three chambers with different visual and tactile cues. The two test chambers (28×22×21 cm) had black walls and parallel bar flooring or white walls and metal grid flooring and were separated by a central compartment (13×22×21) with grey walls and a smooth, grey Plexiglas floor. The three chambers were connected by guillotine doors in colors matching the chamber with which they were attached and males were confined to one chamber or allowed to move freely between chambers.

Experimental design. On day 1 (pre-test) males ($n=5$; paired males) were allowed free access to the entire apparatus for 15 min and the initial preference for each animal was determined. As a group, animals did not display preference for one chamber, but each animal had a slight (less than 60 s) preference (Pitchers et al., 2010; Tenk et al., 2009). On days 2 and 3 (conditioning trials), males mated to one ejaculation in the home cage and mating behavior was observed and recorded (Agmo, 1997). A receptive female was placed in the home cage and total numbers of mounts and intromissions as well as latencies to first mount and intromission (time from presentation of a receptive female to first mount or intromission) as well as latency to ejaculation (time from the first intromission to ejaculation) were recorded during each trial. Immediately following ejaculation males were placed into the paired (initially non-preferred) chamber for 30 min. For control pairings, males were placed into the unpaired (initially preferred) chamber without mating for 30 min. Half of the animals received sex pairing on day 2 and control pairing on day 3. The remaining animals received sex pairing on day 3 and control pairing on day 2. On day 4, a post-test procedurally identical to the pre-test was conducted and conditioned preference was determined. Another group of males ($n=5$; unpaired males) served as a control group and were placed in the chambers without mating on both conditioning days. A preference score (percentage of time spent in the sex-paired chamber) and difference score (time spent in the sex-paired chamber minus the time spent in the control chamber) were calculated for each animal and compared using a paired *t*-test, with a 95% confidence level. Indeed, males formed a significant CPP for the sex paired chamber seen as an increased preference score ($p=0.038$) and difference score ($p=0.04$) in the post-test compared to the pre-test following one pairing with ejaculation, confirming previous reports (Straiko et al., 2007; Tenk et al., 2009; Webb et al., 2009), while control males did not form a preference for either chamber.

Tissue processing. One hour following the end of the post-test, males were anesthetised with sodium pentobarbitol (270 mg/mL) and were transcardially perfused with a 0.9% saline solution followed by 500 mL of 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer (PB). Brains were quickly removed and post-fixed for 1 h in the same fixative and transferred to a 20% sucrose solution for cryoprotection. Brains were sectioned into 35 μm coronal sections on a freezing microtome (Microm, Walldorf, Germany) and collected in 4 parallel series in cryoprotectant solution (30% sucrose in 0.1 M PB containing 30% ethylene glycol and 0.01% sodium azide). Brains were stored at −20 °C until further processing.

Immunohistochemistry: cFos/Orexin

Incubations were performed with gentle agitation at room temperature. Free floating sections were extensively washed in 0.1 M saline-buffered sodium phosphate (PBS). Sections were blocked for 10 min in 1% H_2O_2 (30% stock solution) in PBS, then again rinsed extensively with PBS. Sections were then incubated for 1 h in an incubation solution (PBS containing 0.1% bovine serum albumin and 0.4% Triton X-100). Primary antibody incubations were performed overnight at room temperature in the same incubation solution. Following staining sections were rinsed extensively with PBS, mounted onto plus charged glass slides and coverslipped with dibutyl phthalate xylene (DPX).

One series of sections was immunoprocessed for cFos and orexin. Sections underwent an overnight incubation with a rabbit raised antibody recognizing cFos (rabbit anti-cFos, SC52; 1:10,000, Santa Cruz Biotechnology, Santa Cruz, CA) which was followed by incubation with biotinylated goat anti-rabbit (1:500, Vector Laboratories, Burlingame, CA) for 1 h and a 1-h incubation with avidin horseradish peroxidase complex (1:1000, ABC kit—Vector Laboratories, Burlingame, CA). Finally, sections underwent incubation for 10 min in 0.02% diaminobenzidine (DAB) (Sigma, St. Louis, MO) in 0.1 M PB containing 0.012% hydrogen peroxide and 0.08% nickel sulfate, which resulted in a blue-black reaction product. Next, sections were incubated overnight with a rabbit raised antibody recognizing orexin-A (rabbit anti-orexin-A, H-003-30; 1:20,000, Phoenix Pharmaceuticals, Burlingame, CA) followed by 1-h incubation with biotinylated goat anti-rabbit and ABC, as described above. Immunoreactivity was visualized by a 10-min incubation with 0.02% DAB in 0.1 M PB containing 0.012% hydrogen peroxide, resulting in a reddish brown reaction product.

All antibodies were previously characterized (Chen et al., 1999; Satoh et al., 2004; Solomon et al., 2007). Controls for immunohistochemistry included: primary antibody omission, western blot analysis demonstrating bands of appropriate weight (cFos) and loss of immunohistochemical signal following lesions of orexin neurons with orexin-saporin (orexin).