Hormones and Behavior 58 (2010) 397–404

Contents lists available at ScienceDirect

Hormones and Behavior

journal homepage: www.elsevier.com/locate/yhbeh

Orexin mediates initiation of sexual behavior in sexually naive male rats, but is not critical for sexual performance

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ARTICLE INFO

Article history:
Received 13 April 2010
Revised 3 June 2010
Accepted 4 June 2010
Available online 10 June 2010

Keywords:
Orexin
Hypocretin
Sexual behavior
Copulation
Neural activation
Motivation
Hypothalamus
Novelty
Arousal
Anxiety

ABSTRACT

The hypothalamic neuropeptide orexin mediates arousal, sleep, and naturally rewarding behaviors, including food intake. Male sexual behavior is altered by orexin receptor-1 agonists or antagonists, suggesting a role for orexin-A in this naturally rewarding behavior. However, the specific role of endogenous orexin-A or B in different elements of male sexual behavior is currently unclear. Therefore, the current studies utilized markers for neural activation and orexin cell-specific lesions to test the hypothesis that orexin is critical for sexual motivation and performance in male rats. First, cFos expression in orexin neurons was demonstrated following presentation of a receptive or non-receptive female without further activation by different elements of mating. Next, the functional role of orexin was tested utilizing orexin-B conjugated saporin, resulting in orexin cell body lesions in the hypothalamus. Lesions were conducted in sexually naive males and subsequent sexual behavior was recorded during four mating trials. Lesion males showed shortened latencies to mount and intromit during the first, but not subsequent mating trials, suggesting lesions facilitated initiation of sexual behavior in sexually naive, but not experienced males. Likewise, lesions did not affect sexual motivation in experienced males, determined by runway tests. Finally, elevated plus maze tests demonstrated reduced anxiety-like behaviors in lesioned males, supporting a role for orexin in anxiety associated with initial exposure to the female in naive animals. Overall, these findings show that orexin is not critical for male sexual performance or motivation, but may play a role in arousal and anxiety related to sexual behavior in naive animals.

Introduction

Orexin, also known as hypocretin, is a hypothalamic neuropeptide critical for feeding behavior (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, 2007; Furlong & Carrive, 2007; Furlong et al., 2009; Carter et al., 2009). Orexin neurons are localized to the lateral hypothalamic area (LHA) and perifornical dorsomedial hypothalamus (PFA-DMH) and produce two neuropeptides, orexin-A and B (de Lecea et al., 1998; Sakurai et al., 1998). Orexin neurons have been shown to project to brain structures involved in mediation of arousal including the locus coeruleus, tuberomammillary nucleus and peduculopontine tegmental nucleus (Peyron et al., 1998; Hagan et al., 1999; Horvath et al., 1999; Baldo et al., 2003). Orexin has also been implicated in reward and motivation, specifically related to food and drugs of abuse (Aston-Jones et al., 2009a; b) and orexin neurons have been shown to project to reward-related brain structures in the mesolimbic system including the ventral tegmental area (VTA) and nucleus accumbens (NAc) (Peyron et al., 1998; Fadel & Deutch, 2002; Martin et al., 2002; Baldo et al., 2003). Orexin neurons are activated by conditioned contextual cues associated with food and drug reward (Harris et al., 2005; de Lecea et al., 2006; Choi et al., 2010) and have been shown to play a role in reward-based feeding behavior (Choi et al., 2010). Moreover, intracerebroventricular (ICV) or intraperitoneal administration of an orexin receptor 1 (ORX1) antagonist results in reduced motivation for palatable food (Thorpe et al., 2005; Nair et al., 2008), whereas ICV orexin-A administration can reinstate this motivation (Boutrel et al., 2005).

The role of orexin in other rewarding behaviors is currently unclear, although several studies have implicated a role for orexin in control of sexual behavior in male rats. It has previously been shown that orexin neurons are activated by copulation in male rats (Muschamp et al., 2007). In addition, administration of orexin-A into the medial preoptic area (mPOA) resulted in enhanced sexual performance evidenced by reduced latencies to mount and intromit, and increased frequencies of mounts and intromission (Gulia et al.,

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0018-506X/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.yhbeh.2010.06.004

2003). In contrast, ICV administration of orexin-A attenuated sexual motivation by reducing female preference, although only in highly sexually motivated males (Bai et al., 2009). Studies using ORX1 antagonists have also demonstrated contradictory data, as systemic administration of ORX1 antagonist slightly impaired sexual performance by increasing latency to intromit without affecting other parameters of sexual behavior (Muschamp et al., 2007), while ICV administration of ORX1 antagonist had no effect on sexual motivation (Bai et al., 2009). Together these studies suggest that administration of exogenous orexin-A affects sexual performance and motivation; however, endogenous orexin may not play an important role in mediating sexual behavior (Bai et al., 2009). Therefore, the goal of the present study was to determine if endogenous orexin is essential for male rat sexual motivation and performance.

First, it was determined when during sexual behavior orexin neurons are activated, testing the hypothesis that orexin neurons are activated upon introduction of the rewarding stimulus. Moreover, as it has been shown that sexual experience influences sexual performance (Dewsbury, 1969) and the rewarding properties of sexual behavior (Tenk et al., 2009), it was determined whether sexual experience influences orexin neuron activation during mating. Finally, it was tested whether orexin plays a critical role in sexual motivation and performance using cell body specific lesions of orexin neurons.

Materials and methods

Adult male Sprague–Dawley rats (200–250 g) were obtained from Harlan (Indianapolis, IN) or Charles River Laboratories (Sherbrooke, Quebec, Canada) and housed individually or in pairs depending on the individual experiment (see below) in Plexiglas cages. The colony room was maintained on a 12/12 reversed light–dark cycle (lights off at 10 am) and food and water were available *ad libitum* 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 implanted subcutaneously with 5% 17-β-estradiol benzoate silastic capsules. Sexual receptivity was induced by subcutaneous injections of progesterone (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 conformed to the guidelines outlined by the National Institute of Health and the Canadian Council on Animal Care. All behavioral testing was conducted during the first half of the dark phase under dim red illumination, except when noted otherwise.

Experimental design

cFos expression studies

Male rats (n = 48) were housed individually and half of the animals gained sexual experience in the home cage during 5 twice weekly mating sessions. Mating tests were performed in the home cage to eliminate arousal and cFos expression induced by exposure to a different mating arena and exposure to conditioned cues associated with prior mating (Balfour et al., 2004). A receptive female was introduced into the home cage and males were allowed to mate until one ejaculation or for 60 min. During each test sexual behavior was observed. The total number of mounts and intromissions, as well as the latencies to first mount and intromission (the time from presentation of the receptive female to the first mount or intromission), and ejaculation (the time from the first intromission to ejaculation), was recorded (Agmo, 1997). The remaining half of the animals remained sexually naive. These animals were housed in the same room as the sexually experienced males, were handled and exposed to odors and sounds associated with mating, however did not mate. Naive and experienced animals were each further subdivided into 6 experimental groups (n = 4 per group). The 6 naive and experienced groups included: control males with no exposure to sexual behavior (home cage); males exposed to a non-receptive female in the home cage for 15 min (anestrous female). Males could investigate and interact, however did not mate due to lack of female receptivity; males exposed to the smells of a receptive female placed in a wire mesh box on top of the home cage for 15 min (estrous female); males that displayed mounts, but not intromissions or ejaculation with vaginally masked females (mount); males that displayed mounts and intromissions only (intromission); and males that mated to one ejaculation (ejaculation). One hour after the end of the test, males were sacrificed to analyze cFos expression. Sexually experienced groups were matched on parameters of sexual behavior and there were no significant differences between groups prior to the final test. Moreover, there were no significant differences between naive and experienced groups in numbers of mounts plus intromissions during the final test.

Perfusions: cFos expression

All males were deeply anaesthetised with sodium pentobarbitol (270 mg/mL) and were transcardially perfused with 4% paraformaldehyde (500 mL; PFA). Following perfusion brains were removed immediately and post-fixed for 1 h in the same fixative then transferred to 20% sucrose solution for cryoprotection. Brains were sectioned on a freezing microtome (Microm, Walldorf, Germany) in coronal sections of 35 μm 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) and stored at −20 °C until further processing.

Immunohistochemistry

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

cFos/orexin

One series of sections was immunoprocessed for cFos and orexin. Sections were incubated overnight with a rabbit-raised antibody recognizing cFos (rabbit anti-cFos, sc-52; 1:10000, Santa Cruz Biotechnology, Santa Cruz, CA) followed by 1 hour incubations with biotinylated goat anti-rabbit (1:500, Vector Laboratories, Burlingame, CA) and an avidin horseradish peroxidase complex (1:1000, ABC kit, Vector Laboratories, Burlingame, CA). Sections were incubated for 10 min in 0.02% diaminobenzidine (DAB) (Sigma, St. Louis, MO) in 0.1 M phosphate buffer (PB) containing 0.012% hydrogen peroxide and 0.08% nickel sulfate, resulting in a blue-black reaction product. Sections were then 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 a 1 hour incubation with biotinylated goat anti-rabbit and ABC, as described above. Finally, the sections were incubated for 10 min with 0.02% DAB in 0.1 M PB containing 0.012% hydrogen peroxide, resulting in a reddish brown reaction product.

All antibodies have been characterized previously (Chen et al., 1999; Satoh et al., 2004; Solomon et al., 2007). Immunohistochemical controls included omission of primary antibodies, western blot analysis demonstrating single bands at appropriate weight (cFos), and loss of immunohistochemical orexin signal with orexin-B-saporin lesions (orexin).