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Orexin/hypocretin receptor 1 signaling mediates Pavlovian cue-food conditioning and extinction

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HIGHLIGHTS

- Investigated orexin necessity in cue-food acquisition and extinction learning
- Orexin receptor blockade impaired consolidation and recall during acquisition.
- Orexin receptor blockade impaired consolidation during extinction.

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ABSTRACT

Learned food cues can drive feeding in the absence of hunger, and orexin/hypocretin signaling is necessary for this type of overeating. The current study examined whether orexin also mediates cue-food learning during the acquisition and extinction of these associations. In Experiment 1, rats underwent two sessions of Pavlovian appetitive conditioning, consisting of tone-food presentations. Prior to each session, rats received either the orexin 1 receptor antagonist SB-334867 (SB) or vehicle systemically. SB treatment did not affect conditioned responses during the first conditioning session, measured as food cup behavior during the tone and latency to approach the food cup after the tone onset, compared to the vehicle group. During the second conditioning session, SB treatment attenuated learning. All groups that received SB, prior to either the first or second conditioning session, displayed significantly less food cup behavior and had longer latencies to approach the food cup after tone onset compared to the vehicle group. These findings suggest orexin signaling at the 1 receptor mediates the consolidation and recall of cue-food acquisition. In Experiment 2, another group of rats underwent tone-food conditioning sessions (drug free), followed by two extinction sessions under either SB or vehicle treatment. Similar to Experiment 1, SB did not affect conditioned responses during the first session. During the second extinction session, the group that received SB prior to the first extinction session, but vehicle prior to the second, expressed conditioned food cup responses longer after tone offset, when the pellets were previously delivered during conditioning, and maintained shorter latencies to approach the food cup compared to the other groups. The persistence of these conditioned behaviors indicates impairment in extinction consolidation due to SB treatment during the first extinction session. Together, these results demonstrate an important role for orexin signaling during Pavlovian appetitive conditioning and extinction.

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1. Introduction

The motivation to seek and consume food is essential for survival. One neural substrate mediating this motivation is the neuropeptide orexin/hypocretin (for reviews, see [1–3]), which is synthesized within the lateral hypothalamus [4,5], a brain region critical for feeding [6,7]. Specifically, orexin-A is important for appetitive motivation [1] and binds to both orexin receptors, orexin 1 (OX1R) and orexin 2 receptors; however, OX1R has a higher affinity for orexin-A than for orexin-B [5,8]. Indeed, manipulations that disrupt OX1R signaling interfere with the consumption of standard chow [9–11], as well as binge eating for highly palatable foods [12]. OX1R blockade decreases the motivation to work for and seek high fat food [9,13–15], sucrose [16,17], and saccharin [18]. Similarly, orexin knockout mice consume smaller amounts of sucrose [19] and are less motivated to work for food [15]. These studies clearly demonstrate orexin is necessary for the motivation to obtain food.

However, food consumption is not only driven by internal, physiological signals, but can also be induced by external, environmental signals through associative learning. Cues previously associated with food can later increase the motivation to obtain and consume food independent of physiological hunger across species [20–24]. We recently demonstrated that such non-homeostatic, cue-driven consumption also requires orexin signaling [25]. Additionally, orexin neurons are recruited during late Pavlovian cue-food conditioning when cues reliably signal food delivery [26], and by environmental cues previously associated with food [9,27,28]. Nevertheless, whether orexin signaling is necessary during the initial formation of cue-food associations remains unknown.

Here, we used Pavlovian appetitive conditioning to examine if orexin mediates the initial cue-food acquisition and the extinction of these associations. Employing a pharmacological approach, we systemically blocked OX1Rs with the selective antagonist SB-334867 (SB) during the two initial sessions of either acquisition or extinction in two separate experiments. Using a crossover design, we monitored learning in subjects that received either vehicle or SB prior to one or both sessions. This approach allowed assessment of the role of orexin during various phases of learning – the initial acquisition, the consolidation phase, and the recall of the memory. Furthermore, acquisition and extinction are expressed through different behaviors, an increase in responding to a reward and a decrease in responding in the absence of a reward, respectively. Thus, examination of both types of learning allowed for an assessment of orexin signaling function in learning independent of the direction of the behavior and whether the reward was present or not.

2. Materials and methods

2.1. Subjects

Sixty-four, experimentally naïve, male Long-Evans rats (300–325 g) obtained from Charles Rivers Laboratories were used. Rats were individually housed and maintained on a 12 h light/dark cycle (lights on at 06:00). Behavioral testing was conducted during the light phase between 09:00 and 13:00. Rats were given one week to acclimate to the colony room with ad libitum access to water and food (standard laboratory chow) and were handled and weighed daily. All experiments were in accordance with the National Institutes of Health *Guidelines for Care and Use of Laboratory Animals* and were approved by the Boston College Institutional Animal Care and Use Committee.

2.2. Apparatus

Habituation, acquisition, and extinction occurred in the same set of identical behavioral chambers (30 × 28 × 30 cm; Coulbourn Instruments, Allentown, PA), located in a room different from the colony housing room. Behavioral chambers were composed of an aluminum top and sides with one side containing a recessed food cup (3.2 × 4.2 cm), a transparent Plexiglas front with a hinge, a transparent Plexiglas back, and a black Plexiglas floor, and were illuminated with a house light (4 W). Each chamber was contained in an isolation cubicle (79 × 53 × 53 cm; Coulbourn Instruments, Allentown, PA) composed of monolithic rigid foam walls, which contained a ventilation fan (55 dB). A video camera located on the rear wall of each isolation cubicle recorded subjects' behavior during the sessions. The conditioned stimulus (CS) was a 10 s tone (75 dB, 2 kHz), and the unconditioned stimulus (US) was two food pellets (formula 5TUL, 45 mg: Test Diets, Richmond, IN) delivered into the food cup. A computer located in an adjacent room controlled the stimuli and video cameras (GraphicState 3.0, Coulbourn Instruments, Allentown, PA).

2.3. Drugs

SB-334867 (SB; Tocris Bioscience, Ellisville, MO, USA) was suspended in a solution consisting of 2% dimethylsulfoxide and 10% 2-hydroxypropyl-*b*-cyclodextrin (Sigma-Aldrich, St. Louis, MO, USA) in sterile water. SB was administered via intraperitoneal injection (i.p.) at a volume of 2 ml/kg and concentration of 20 mg/kg. SB or vehicle was given 30 min prior to each acquisition session (Experiment 1) or prior to each extinction session (Experiment 2).

2.4. Experiment 1: effect of SB on the acquisition of Pavlovian appetitive conditioning

Experimental design is shown in Fig. 1A. Rats were food restricted to gradually reach 85% of their ad libitum body weight, which was maintained throughout the experiment. Prior to acquisition, rats were given one 30 min habituation session to acclimate them to the behavioral chambers. During that session all subjects had access to 1 g of the food pellets (US) in the food cup to familiarize them with the pellets.

Acquisition training commenced the following day. All groups received two identical acquisition sessions on two separate days. During