Physiology & Behavior xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Physiology & Behavior

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

Deletion of Melanin Concentrating Hormone Receptor-1 disrupts overeating in the presence of food cues

Andrew Sherwood [a], Peter C. Holland [a], Antoine Adamantidis [b], Alexander W. Johnson [c,*]

[a] Department of Psychological and Brain Sciences, Johns Hopkins University, Baltimore, MD 21218, United States
[b] Douglas Hospital Research Centre, Department of Psychiatry, McGill University, 6875 LaSalle Boulevard, Montreal, Canada
[c] Department of Psychology and Neuroscience Program, Michigan State University, East Lansing, MI 48824, United States

HIGHLIGHTS

- Overeating in the presence of food cues was examined.
- MCH-1R knockout mice were tested for cue potentiated feeding.
- Deletion of MCH-1R disrupted cue potentiated feeding.
- This disruption reflected reduction in size and number of licking bursts.

ARTICLE INFO

Article history:
Received 13 March 2015
Received in revised form 9 May 2015
Accepted 29 May 2015
Available online xxxx

Keywords:
Lateral hypothalamus
Licking microstructure
Binge eating
Obesity
Glucose sensing
Allostasis

ABSTRACT

Exposure to environmental cues associated with food can evoke eating behavior in the absence of hunger. This capacity for reward cues to promote feeding behaviors under sated conditions can be examined in the laboratory using cue-potentiated feeding (CPF). The orexigenic neuropeptide Melanin Concentrating Hormone (MCH) is expressed throughout brain circuitry critical for CPF. We examined whether deletion of the MCH receptor, MCH-1R, would in KO mice disrupt overeating in the presence of a Pavlovian CS + associated with sucrose delivery. While both wild-type controls and KO mice showed comparable food magazine approach responses during the CPF test, MCH-1R deletion significantly impaired the ability of the CS + to evoke overeating of sucrose under satiety. Through the use of a refined analysis of meal intake, it was revealed that this disruption to overeating behavior in KO mice reflected a reduction in the capacity for the CS + to initiate and maintain bursts of licking behavior. These findings suggest that overeating during CPF requires intact MCH-1R signaling and may be due to an influence of the CS + on the palatability of food and on regulatory mechanisms of peripheral control. Thus, disruptions to MCH-1R signaling may be a useful pharmacological tool to inhibit this form of overeating behavior.

1. Introduction

The obesogenic environment is characterized by a sedentary lifestyle and the availability of energy dense foods that can be acquired at little cost [1]. However, the detrimental effects of this environment are proving costly for our society as it contributes to weight gain, obesity and associated co-morbidities (e.g., heart disease and diabetes) [2]. This affects not only obese individuals whose quality of life is severely reduced, but also society in general, where in the US alone associated annual healthcare costs are estimated to be in excess of $190 billion [3]. At the same time, there is a lack of available pharmacotherapeutic strategies to aid in reducing body weight in obese individuals [4]. Thus, there is a critical need to identify the variables that influence overeating of food and the underlying brain mechanisms controlling this behavior.

Food-associated cues (e.g., television advertisements, radio jingles, and catchy signboards) likely contribute to eating by altering food preferences and enhancing consumption [5], which may promote weight gain and obesity. In the laboratory it is possible to examine the influence of food cues on eating behaviors using cue-potentiated feeding (CPF), where external cues paired with food delivery lead to significant overeating behavior under non-deprived conditions [5,6]. This learned overeating response has been revealed in mice [7], rats [6,8], and humans [9,10], with foods of varying degrees of nutrition and palatability. CPF has been shown to depend on limbic and prefrontal circuitry that includes the lateral hypothalamus (LH) [11], basolateral amygdala (BLA) [6], ventral hippocampus (VH) [12] and ventromedial prefrontal cortex (vmPFC) [13].

Due to its synthesis in the LH [14,15] and projections through both CPF and classical reward circuits [16], the central feeding peptide Melanin

* Corresponding author at: Department of Psychology, Michigan State University, East Lansing, MI 48824, United States.
E-mail address: awj@msu.edu (A.W. Johnson).

http://dx.doi.org/10.1016/j.physbeh.2015.05.037
0031-9384/

Concentrating Hormone (MCH) may play a critical role in influencing eating in the presence of reward cues. MCH exerts its physiological effects by binding to and activating the G protein-coupled MCH receptors, MCH-1R and MCH-2R. While in several species (e.g., primates, dogs and ferrets) the action of MCH-2R is preserved [17], in rodents it is either absent or non-functional [18]. MCH is upregulated during periods of food withdrawal or in hypoleptinemic *ob*/*ob* mice [19,20], and elicits food intake when infused centrally [21,22]. Transgenic overexpression of MCH also leads to hyperphagia and weight gain [23], whereas deletion or antagonism of MCH-1R suppresses intake [24,25]. With respect to reward learning, MCH influences both food-seeking and cocaine-seeking [26–28], and deletion of MCH-1R disrupts conditioning of incentive motivation to a reward-paired auditory cue, leading to reductions in its ability to promote novel instrumental nose-poke responding [29].

Given this expression in CPF circuitry and its role in the regulation of food intake and reward learning, we hypothesized that MCH would play a significant role in CPF. Here we used a lack-of-function approach through MCH-1R gene knockout (KO) mice [18]. Under food-deprived conditions, mice were trained to acquire a simple Pavlovian discrimination followed by ad-libitum access to lab chow for a period of ≥3 days. After this satiety treatment, we examined the ability of food cues to promote overeating under non-deprived conditions. Furthermore, we used microstructure analyses to examine the variables that may underlie any changes in consummatory behavior (e.g., orosensory positive feedback and/or conditioned negative feedback) [30–32].

2. Methods

2.1. Subjects

The inactivation of the MCH-1R allele and the generation of KO animals and the genotyping method have been previously described [33]. Heterozygous MCH-1R$^{+/-}$ mice were backcrossed a minimum of eight times to the C57BL/6J strain (Jackson Laboratory, Bar Harbor, ME, USA). Seventeen WT and thirteen KO mice were used and were tested at approximately 3 months old, and were housed three or four to a cage under a 12 h light/dark cycle (lights on at 07:00–19:00 h). Food deprivation began at least 2 days prior to the start of the experiment by restricting access to two daily meal pellets. Behavioral training and testing were completed in the light cycle between 09:00 and 17:00 h and were conducted under the auspices of the Johns Hopkins University Institutional Animal Care and Use Committee.

2.2. Apparatus

All behavioral procedures were conducted in six individual chambers (53 × 35 × 35 cm LWH) with aluminum front and back walls, clear polycarbonate sides, and a floor made of 17.8-mm stainless steel rods spaced 0.5 cm apart (Med Associates, St Albans, VT, USA). Each chamber was contained in a custom-built sound attenuating box with tubing connecting to solenoids located on the outside of the sound attenuating box. Fluid rewards could be delivered, via solenoid activation, to a 50 μL food well that was housed in a food magazine and recessed in the center of one end of the chamber. The food well contained a custom-built lickometer through which fiber optics was used to introduce a light beam through the fluid–air interface of a fluid bolus. Through this approach, individual time-stamped licks were detected as disturbances in the light surface at the interface when the fluid was contacted. A vacuum was attached to the bottom of the food well, which could be released via an attached solenoid. An infrared photocell placed inside the magazine monitored the time spent and number of head entries made into it. An audio generator, which could be programmed to emit a 3 kHz tone or white noise (each 80 dB), was mounted on the outside of the chamber on the wall opposite the magazine. Chamber illumination was provided by a 28 V, 100 mA house light mounted on the inside wall of the sound-attenuating chamber. An IBM-compatible computer equipped with Med-PC software (Med Associates, St. Albans, VT, USA) controlled and recorded all stimuli and responses.

2.3. Procedure

Prior to training mice received at least two days of food deprivation, and once they reached ~90% of their baseline weights the magazine training commenced. Mice were placed in their assigned testing chambers with a 10% sucrose (*w*/*v*) reward immediately available in the food well. Upon entering the food magazine the mouse was free to consume its reward, initiating the first of 60 trials. The inter-trial interval between reward deliveries varied randomly on a random-time 30 s schedule with a full session of 60 trials taking approximately 30–45 min. At the conclusion of the second day of the magazine training, any subject that did not have at least 10 s of time in the magazine with the reward present (US time) was given additional training in order to reach this criterion.

Following the food magazine training, mice received 16 days of Pavlovian conditioning. During each Pavlovian conditioning session, mice received a total of 20 trials: 10 reinforced trials and 10 non-reinforced trials. Each trial consisted of a 20 second presentation of either a tone or white noise stimulus divided into four 5 s epochs. For the reinforced CS + trials, a 10% sucrose reward was delivered twice during two of the four epochs, with the constraint that for the first 5 s epoch, sucrose delivery could occur on a maximum of three and a minimum of two (out of the ten) trials. Subsequently, CS + responses from the seven trials where sucrose was not delivered during the first 5-s epoch were used as our measure of conditioning. Given this measure was uncontaminated by US delivery, it more appropriately reflected conditioning to the CS. The pseudo-random reward schedule for the epochs was changed every third day. Any reward not consumed was removed at the end of the trial via the vacuum situated at the bottom of the well. During a CS − trial, no reward was delivered. For data presentation, CS − responses during the first 5-s epoch, from seven of the ten trials (chosen at random) were used. Which stimulus was reinforced, tone or noise, was counterbalanced across the two groups. Initially, a dummy solenoid was paired with the reward delivery to facilitate consumption of the reward and acquisition of the stimulus–reward pairing, but this additional cue was removed after the third day of training.

At the completion of 16 Pavlovian conditioning sessions, subjects were given 2 days (3 nights) of ad-libitum access to their standard laboratory diet with the goal of restoring subjects to at least 100% of their original baseline weight. Subjects were then tested for CPF with the CS + and CS −, each tested individually on separate days, with the order of tests fully counterbalanced across and within groups of mice. The two potentiated feeding test sessions examined the effects of each stimulus on consumption: the sucrose reward was available for consumption at all times for both tests. At the start of the session, 50 μL of sucrose was available in the food cup, and additional 50 μL deliveries occurred every 40 licks as mice consumed the liquid. Test sessions began with a 2-min baseline period. This was followed by one of 4 test trials during which either the tone or the noise stimulus was presented for 20 s. Each trial was then followed by a 2-min interval before the onset of the next 20 s trial, or the end of the test session.

2.4. Data analyses

The Pavlovian training data (magazine responses/min) were analyzed with a mixed ANOVA with within subject variables of cue (CS+, CS-) and session block (1-4), and between-subject variable of group (WT, KO). For the CPF test the rate of licking (licks/min) and the size and number of licking bursts were also examined. A licking burst was defined as two or more consecutive licks, with pauses greater than 1 s determining the licking burst termination. The burst number reflected the initiation of licking behavior following a 1 s pause. We previously conducted an extensive parametric study supporting the suitability of the

Please cite this article as: A. Sherwood, et al., Deletion of Melanin Concentrating Hormone Receptor-1 disrupts overeating in the presence of food cues, Physiol Behav (2015), http://dx.doi.org/10.1016/j.physbeh.2015.05.037