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Food-induced reinforcement is abrogated by the genetic deletion of the MT_1 or MT_2 melatonin receptor in C3H/HeN mice

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ABSTRACT

Palatable food is known for its ability to enhance reinforcing responses. Studies have suggested a circadian variation in both drug and natural reinforcement, with each following its own time course. The goal of this study was to determine the role of the MT_1 and MT_2 melatonin receptors in palatable snack food-induced reinforcement, as measured by the conditioned place preference (CPP) paradigm during the light and dark phases. C3H/HeN wild-type mice were trained for snack food-induced CPP at either ZT 6 – 8 (ZT: Zeitgeber time; ZT 0 = lights on), when endogenous melatonin levels are low, or ZT 19 – 21, when melatonin levels are high. These time points also correspond to the high and low points for expression of the circadian gene *Period1*, respectively. The amount of snack food (chow, Cheetos®, Froot Loops® and Oreos®) consumed was of similar magnitude at both times, however only C3H/HeN mice conditioned to snack food at ZT 6 – 8 developed a place preference. C3H/HeN mice with a genetic deletion of either the MT_1 (MT_1KO) or MT_2 (MT_2KO) receptor tested at ZT 6 – 8 did not develop a place preference for snack food. Although the MT_2KO mice showed a similar amount of snack food consumed when compared to wild-type mice, the MT_1KO mice consumed significantly less than either genotype. We conclude that in our mouse model snack food-induced CPP is dependent on time of day and the presence of the MT_1 or MT_2 receptors, suggesting a role for melatonin and its receptors in snack food-induced reinforcement.

1. Introduction

Obesity is a growing health problem within the United States, with over 30% of adults displaying an obese phenotype [1] characterized by a body mass index greater than 30 [2]. Studies have suggested a potential role for "food addiction" as a component of obesity [3,4]. This push toward classifying certain types of excessive food intake as "food addiction" is supported by studies demonstrating food, particularly palatable foods, interact with the brain in a similar manner to drugs of abuse [2,5–7].

A reinforcer is a stimulus, which increases the probability of an associated behavior occurring again. Food is considered to be a rewarding/reinforcing substance [8,9], as evidenced by the fact that mice and rats will work to obtain food in an operant conditioning paradigm and food-paired with a distinct contextual cues will result in the formation of a place preference. The rewarding/reinforcing properties of food can be altered by taste, texture, and/or palatability [10]. For humans one type of food considered to have elevated reinforcing properties are "snack foods," which refers to a subset of commercially produced highly palatable foods [11]. Using the definition of "snack foods" proposed by Hess et al., these foods are "energy-dense, nutrient-poor foods high in sodium, sugar, and/or fat" [12]. Ingestion of these foods has markedly increased [13,14] and is suggested to be a contributing factor in the elevated incidence of obesity [11,15]. The brain reward pathways play an important role in food intake behavior, particularly the dopamine system and μ opioid system [2]. Within the reward pathway food and food-related cues activate numerous areas including the striatum and ventral tegmental area, as well as other structures [16–18]. In contrast food and food-related cues decrease activation of the lateral and medial habenula [18].

Recent studies have demonstrated that while both natural reinforcement and drug reinforcement display a diurnal variation, they exhibit different times of maximum and minimum sensitivity [19]. This suggests the reward pathway may be preferentially primed for natural *vs*. drug reinforcement at various times, which in turn suggests susceptibility to regulation by a circadian hormone and/or molecule. The circadian molecule, melatonin, is released from the pineal gland with elevated levels at night and the low levels during the day [20]. Melatonin exerts its effects through action at two G protein-coupled receptors, termed MT_1 and MT_2 [21]. Studies in our laboratory have

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linked deletion of either the MT_1 (MT_1KO) or MT_2 (MT_2KO) melatonin receptor to a complete abrogation of methamphetamine-induced reinforcement, as quantified by the conditioned place preference paradigm (CPP) [22]. Accordingly, the melatonin receptors are located throughout the brain including areas of the reward pathway such as the nucleus accumbens [23], ventral tegmental area [23], substantia nigra reticulate [24], hippocampus [25–27], and habenula [28]. Furthermore, suppression of melatonin has been linked to obesity and metabolic syndrome [29], suggesting melatonin may be a prime candidate for modulating food intake and reinforcement.

The goal of this study was first to assess the potential diurnal variation in snack food-induced CPP and the role of the melatonin receptors (MT_1 and MT_2) in this behavior. The CPP paradigm works as a model of classical conditioning by pairing a reinforcing stimulus with a previously neutral set of environmental cues, in the form of wall color and floor texture. Following multiple pairings, the reinforcing properties of the stimulus are transferred to the previously neutral environmental cues resulting in increased time spent in the compartment where the reinforcer was provided [30]. This paradigm has frequently been used to assess the reinforcing properties of drugs of abuse [19,22,30] as well as natural reinforcement such as food [11,31,32].

Here, we demonstrate snack food-induced place preference in C3H/HeN mice was dependent on time of day and on the presence of the MT_1 and MT_2 melatonin receptors. Snack food induced a statistically significant place preference in wild-type mice when provided during the light period (ZT 6 – 8), however this preference was abrogated during the dark period (ZT 19 – 21), when melatonin levels are elevated. Furthermore, deletion of either melatonin receptor (MT_1 or MT_2) resulted in a complete abrogation of place preference during the light phase.

2. Materials and methods

2.1. Animals and husbandry

C3H/HeN (104 males) mice were bred and maintained in the Laboratory Animal Facility at the University at Buffalo. Wild-type C3H/HeN mice and C3H/HeN mice homozygous for receptor deletions of the MT_1 melatonin receptor (MT_1KO) or MT_2 melatonin receptor (MT_2KO) were generated in our former laboratory at Northwestern University by backcrossing C57Bl/6J MT_1KO mice (donated by Dr. Steven Reppert; Massachusetts General Hospital, Boston, MA, USA) and C3H/HeN MT_2KO mice (donated by Dr. Steven Reppert; Massachusetts General Hospital, Boston, MA, USA) [33–35] with C3H/HeN mice (Harlan/Envigo, Indianapolis, IN, USA) as previously described [22,36,37].

Mice were maintained in humidity and temperature-controlled (22 ± 1 °C) rooms with food (Harlan Teklad 2018sx) and water provided *ad libitum* prior to the onset of the experimental protocol. All animal procedures were approved by the University at Buffalo Institutional Animal Care and Use Committee and adhered to the National Institutes of Health guidelines.

Male mice were group-housed (3 – 5 per cage) at weaning and maintained in a 14 h: 10 h light/dark cycle, with 150–200 lux light illumination at the level of the cage. Animals were housed in standard polycarbonate cages (30 × 19 cm) with corncob bedding. To maintain consistency with previous studies in our laboratory examining methamphetamine-induced place preference mice were switched to a 12 h: 12 h light dark cycle within ventilated, light-tight cabinets at 4–7 weeks of age and 10–14 days prior to experiment onset.

2.2. Food preparation

Food was chosen based on previous papers detailing the reinforcing effects through the CPP paradigm [11]. Wild-type mice were randomly assigned to three groups: “chow,” “snack,” or “no food.” The “chow” group received chow only (Harlan Teklad 2018sx); the “snack” group received a mixture of equal parts chow, Oreos ®, Cheetos ® and Froot Loops ®, and the “no food” group did not receive any food during the conditioning session. Foods for the snack food group were chosen based on their ability to induce place preference in the literature [11,32,38]. The nutritional characteristics of the foods used are described in Table 1. Based on the results obtained from the wild-type mice at ZT 6 – 8, all subsequent testing (wild-type mice at ZT 19 – 21, MT_1KO mice at ZT 6 – 8, and MT_2KO mice at ZT 6 – 8) were only divided into the “snack” and “no food” groups.

Table 1
Nutritional comparison of food-types used.

Food Type	kcal/g	Carbohydrate	Fat	Protein	Added Sugar	Sodium
Chow (2018sx)	3.1	44.2%	13.2%	18.6%	–	0.2%
Froot Loops	3.8	85.7%	4.8%	4.8%	33.3%	0.5%
Cheetos	5.7	53.6%	35.7%	7.1%	–	0.9%
Oreos	4.7	73.5%	20.6%	2.9%	41.2%	0.5%

2.3. Video tracking system and apparatus

Mouse location and distance traveled in the CPP apparatus were monitored by TopScan (CleverSys Inc, Reston, VA, USA) video tracking software as previously described [22]. Briefly 8 testing chambers were monitored *via* top-view imaging provided by four Sony video cameras. Chambers were placed upon a light panel with the ability to emit white light (daytime studies: ZT 6 – 8) or infrared light (nighttime studies: ZT 19 – 21). Each chamber consisted of two choice compartments measuring 15 × 15 × 25 cm with distinct contextual cues (wall color and floor texture) and one neutral central compartment measuring 10 × 15 × 25 cm. Compartments were separated *via* guillotine door.

2.4. Experimental design

Experiments were conducted at two time points representing the peak and trough of melatonin production in wild-type C3H/HeN mice (Fig. 1) [39]. In MT_1KO and MT_2KO experiments were only conducted during the light phase, as no diurnal variation in preference was observed in previous studies examining methamphetamine CPP in these mice. Daytime experiments, representing the trough of melatonin production, were performed from Zeitgeber time (ZT) 6 – 8 (ZT 0 = lights on). Nighttime experiments, representing the peak of melatonin production, were conducted from ZT 19 – 21 (Fig. 1). For all experiments mice were moved into the testing room one hour prior to test onset. A new cohort of mice was used for each experiment and treatment group.

One week prior to experiment onset mice were individually housed, with food and water provided *ad libitum*. Mice were weighed daily, for 7 days; this was averaged together to establish the initial baseline weight. Mice were handled and received enough food (chow, Oreos®, Cheetos ® and Froot Loops ®) to maintain 90% free feeding weight, during the three days prior to the initiation of the experimental protocol (Fig. 1). This was done in order to acclimate mice to the experimental food.

The CPP paradigm consisted of nine days of testing (Fig. 1). Day 1 and Day 2 consisted of a 20-min *habituation* and *Pre-CPP* test respectively. *Habituation* was used to eliminate chamber novelty, while *Pre-CPP* was used to establish initial compartment bias. The *conditioning period* took place from Day 3 to Day 8. Mice were confined to one compartment for each 60-min daily conditioning session. Mice were confined to their initially least preferred compartment on Days 3, 5, and 7, as determined by the *Pre-CPP* (Day 2) and received chow, snack (chow, Oreos®, Cheetos®, and Froot Loops®), or no food. Mice were confined to their initially most preferred compartment on Days 4, 6, and 8 and received no food. The amount of food consumed during the