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Emotional profile of female rats showing binge eating behavior

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HIGHLIGHTS

- Binge eating behavior was induced in female rats by providing limited access to margarine.
- Emotional profile of rats were evaluated by mean of behavioral mazes.
- Bingeing rats were less anxious and depressed after margarine consumption
- We provide evidence of an altered emotional states in bingeing female rats.

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ABSTRACT

Binge eating disorder (BED) is characterized by uncontrolled consumption of a large amount of food in a brief period of time. A large body of evidence has shown that BED can be a chronic condition associated with elevated psychiatric comorbidity, including depression and anxiety, and compulsive behavior. In this study we used an animal model of BED in which binge eating behavior was induced in female rats by providing limited access to high fat diet (margarine) to investigate the emotional traits of bingeing animals before and after the binge-like consumption of margarine. Using the plus maze test to disclose a potential anxious phenotype, we found that bingeing rats are much more anxious before the access to margarine, and that this condition is significantly reduced after its consumption. Conversely, no difference was detected between bingeing rats in the marble burying test before and after access to margarine. Yet, the number of marbles buried by bingeing rats before margarine consumption was significantly higher than control groups thus suggesting a compulsive-like trait. In the forced swimming test, bingeing rats showed a decrease in depression-like behavior after the consumption of margarine. Altogether, our findings demonstrate the occurrence of an altered emotional state in female rats showing binge eating behavior.

1. Introduction

According to the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5), binge eating disorder (BED) is now recognized as a specific eating disorder [1]. It is characterized by binge eating episodes, defined as rapid and excessive consumption of food in a short period of time, along with loss of control and psychological distress. Food consumed during binge episodes is typically highly palatable, with high fat or sugar content and consequently rich in calories. In BED, binge episodes are typically not associated with inappropriate compensatory behaviors (such as vomiting, excessive physical activity, and laxative use) aimed to counteract the excessive intake of calories. Therefore, bingeing people incur a higher risk of weight gain which, in the majority of them (approximately 70%), leads to obesity [1–4].

BED has a high social impact. Epidemiological studies revealed that BED is the most widespread eating disorder with an estimated 1.9% lifetime prevalence in the general population [5]. Like other eating disorders, BED is more prevalent in females, with males representing 30–40% of the cases [6].

BED can become a chronic disease characterized by frequent exacerbations or relapses and high psychiatric comorbidity [5,7,8]. A clear link between BED and psychiatric comorbidity is reported by several clinical studies showing that binge eaters have a significant higher rate of major depression [9] and anxiety disorders than people without BED [10–13]. Notably, individuals with psychiatric comorbidities may have a more severe form of BED [14]. It is well documented that binge eating episodes are maintained *via* negative reinforcement, such as reduction of

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negative emotional states [15,16]. As reported by many binge eaters, ingestion of large amounts of food, especially highly palatable foods, during binge episodes produces an immediate feeling of well-being and reduces the negative emotional states present before overeating. This feeling of well-being is only temporary as negative states take over again after bingeing thus creating a vicious cycle [16–18]. Further, patients with BED showed increased food-related impulsivity in comparison with weight-matched and normal-weight controls, which may contribute to uncontrolled and excessive food intake and maintenance of binge eating behavior [19].

In order to understand the aberrant eating pattern underlying BED, animal models of binge eating behavior have been developed [20,21]. In fact, intermittent access to highly palatable food can induce compulsive overeating in rodents that remains stable over prolonged periods of time [22–24]. Similar to bingeing humans, animals consumed a large quantity of palatable food in the absence of hunger since they are never food deprived [25]. Although only partially, some of the psychological aspects of BED can be evaluated in animals by means of widely validated behavioral paradigms able to analyze anxiety- and depression like traits, social impairment and obsessive-compulsive behavior [26].

Using the limited access protocol in which binge-type eating is induced in female rats by providing sporadic and time-limited access to an optional source of dietary fat (margarine), this study aimed to characterize the neurobehavioral profile of binge rats before and after the binge-like margarine consumption, in order to make a comparison with the human condition. This characterization included tests of spontaneous locomotor activity, elevated plus-maze behavior (to detect anxiety), marble burying (index of compulsive-like behavior and/or anxiety-like behavior) and forced swimming test (to detect a depression-like phenotype).

2. Materials and methods

2.1. Animals

Forty two female Sprague Dawley rats (Harlan Nossan, Udine, Italy) weighing 185–200 g at the start of the study (60–65 days old) were used in this study. Animals were individually housed in a climate-controlled animal room (21 ± 2 °C temperature; 60% humidity) under a reversed 12 h light/dark cycle (lights on 12:00 a.m.) with standard rat chow and water *ad libitum*. All experiments were approved by the local Animal Care Committee and carried out in strict accordance with the E.C. Regulations for Animal Use in Research (CEE No. 86/609).

2.2. Diets

High-fat diet (Margarine, Gradina Unilever Italia Mkt.): 70% kcal from fat, <1% kcal from carbohydrate, containing 6.5 kcal/g. Standard rat chow (Safe, France): 3% kcal from fat, 61% kcal from carbohydrate, 16% kcal from protein, 0% moisture, containing 2.9 kcal/g.

2.3. Experimental procedures

The general procedure was carried out as described previously [27]. After one week of adaptation, margarine was provided during a single overnight period to prevent neophobia. Rats were then matched by body weight and margarine intake and divided into three groups which correspond to the following diet conditions:

• *Low restriction* (LR): chow and water were available *ad libitum*. In addition, animals were given 2 h (h) access to a separate bowl of margarine introduced into the home cage every day of the week.

• *High restriction* (HR): chow and water were available *ad libitum*. In addition, animals were given 2 h access to a separate bowl of margarine introduced into the home cage on Mondays, Wednesdays and Fridays.

Animals were given access to margarine in the light phase starting 2 h prior to the start of the dark cycle [27].

• *Control* (CTRL): chow and water were available *ad libitum*. Margarine was not provided at any time of the study.

All rats were maintained on their respective diet protocol for the entire study. Margarine and/or standard chow consumed by each diet group were measured on Mondays, Wednesdays and Fridays (MWF) before and after the 2 h period access to margarine. As shown in Fig. 1, once binge-type eating behavior was stable (4th week) behavioral tests started and were completed within four weeks.

2.4. Behavioral tests

Behavioral tests were performed 1.5 h before (pre-binge) and 1.5 h after (post-binge) the 2 h margarine access on Fridays. Animals from each diet group (N = 14 per diet group) were randomly assigned to two different groups to perform tests during the pre-binge (N = 7) and the post-binge phase (N = 7) and were maintained on their respective group for the entire study. Considering that margarine was provided 2 h prior to the start of the dark cycle, in the pre-binge phase, tests were carried out during the light cycle in a room illuminated by a neon lamp (50 Lux), while in the post-binge phase, tests were carried out during the dark cycle in a room dimly illuminated by a red lamp (3 Lux).

2.4.1. Spontaneous locomotor activity (LA)

Apparatus and procedure were as described previously [28]. Rats were individually placed into the center of transparent Plexiglas cages (42 cm × 30 cm × 60 cm) fitted with two sets of 16 photocells located at right angles to each other, projecting horizontal infrared beams 2.5 cm apart and 4 cm above the cage floor and a further set of 16 horizontal beams which height could be adapted to the size of the animals. Cumulative horizontal and vertical movement counts were recorded for 30 min by Digiscan Animal Activity Analyser Software (Omnitech Eletronics, USA) and assessed in 10 min intervals.

2.4.2. Elevated plus maze (EPM)

The apparatus consisted of two opposite closed (50 × 10 × 40 cm) and open (50 × 10 cm) arms forming a plus shaped maze. The structure was elevated to a height of 50 cm from the floor.

All four arms were connected at right angle at a central area where animals were placed (facing an open arm) and were left free to explore the maze for 5 min (300 s). During a 5 min test period, the following behavioral measures were recorded:

- number of open and closed arm entries
- time spent in the open and closed arms.

Closed arms entries were used as a measure of locomotor activity. The percentage (%) of time spent in the open arms [expressed as: time spent in open arms / total time(300 s) × 100] and the percentage (%) of open arms entries [expressed as: open-arms entries / total arm entries × 100] were used as anxiety measures.

2.4.3. Marble burying test (MBT)

The marble burying test was conducted according to Andersen and colleagues [29] with some modifications. Rats were individually placed in a transparent acrylic cage (54 × 20 × 34.5 cm), without food and water. Twenty-four clear glass marbles (1 cm diameter), distributed on top of 5-cm-deep fresh spruce wood bedding, were evenly spaced in six lines along the short wall of the cage. Individual subjects were placed in the test cage and their behavior was monitored for 30 min through an Any-Maze apparatus and video recorded by a camera placed 1.5 m above the cage. The number of marbles buried for at least 2/3 was counted by the experimenters and the results were expressed as the number of marbles covered by sawdust. New bedding was used for each animal, and marbles were cleaned with a 70% ethanol solution between animals.